INTRODUCTION

The consumer’s perception of ‘chemicals in the air (cars, plants, factories, etc.)’ being responsible for skin, scalp and hair problems is globally increasing. In head-to-head comparison for Brazil and US the perception was increasing from 11% to 37% and from 13% to 23%, respectively, from 2012 to 2013. This development leads to a huge demand for anti-air pollution actives with proven efficacy [1].

The consumer’s perception is based also on epidemiologic studies showing that exposure of humans to airborne, traffic related particulate matter (PM) is associated with increased signs of extrinsic skin aging including wrinkle formation and, most importantly, pigment spot formation [2,3,4]. This effect was strongest with coal, indicating that it might be due to carbon particles (with a size in the nanometer/hicrometer range) and/or organic substances bound to the surface of these particles, in particular polyaromatic hydrocarbons (PAH) such as benzo(a)pyrene (BaP).

By using model particles as surrogates for authentic street particulate matter SRM1650b and SRM2975 [5] we observed up-regulation of marker genes indicative for premature aging like matrix metalloproteinase-1 (MMP-1), interleukin 6 (IL-6) and propiomelanocortin (POMC) in adult primary human keratinocytes. These studies clearly showed that the diesel exhaust particles (DEPs) stimulate the premature aging processes inter alia via any hydrocarbon receptor (AhR) pathway as Cyp1A1 gene expression was increased and could be diminished by the known AhR antagonist 3'-methoxy-4'-nitrofluorone (MNF) [6] (results not shown). Also POMC gene upregulation could be diminished by MNF [7] proving again the relevance of inter alia AhR in air pollution induced aging processes.

The aim of this study was to show the protective effect of the recently identified AhR antagonist E2/2-benzylindene-5,6-dimethoxy-3,3-dimethylindan-1-one (BDDI, Fig. 1) [8] against DEP induced premature aging.

MATERIAL AND METHODS

Cell culture: Adult human epidermal keratinocytes were kept sub-confluent in 12well plates for culture and treatment with DEPs. 24h prior to addition of DEPs keratinocytes were starved in keratinocyte medium without BPE and without EGF. The test compound BDDI has been dissolved in DMSO (10mM) and has been applied 2h prior to treatment with the DEPs.

Model DEPs: Standard reference materials (SRMs) SRM1650b and SRM2975 of the National Institute of Standards and Technology, Gaithersburg, MD, USA, were used as appropriate surrogates for authentic street particulate matter. Both SRMs were described in detail before [5] and are well characterized (Tab. 1) [8,10]. The SRMs were suspended in phosphate buffered saline and were sonicated for 1 min and then directly added to the keratinocytes at a concentration of 1.5 µg/ml.

RNA Isolation and PCR: Total RNA was prepared as described earlier [11]. Cyp1A1 and MMP-1 gene were quantified 6h after application of DEPs. IL-6 and POMC gene were quantified 24h after application of DEPs by qRT-PCR. Three samples for each condition have been processed with 2 determinations each.

RESULTS

Cyp1A1 gene expression was significantly increased in adult keratinocytes from a female Caucasian donor aged 53 by 23.23-fold ± 0.64SE and 5.10-fold ± 0.06SE by SRM1650b and SRM2975, respectively, after 24h. BDDI inhibited significantly the upregulation by 96.7% and 88.2%, respectively (Fig. 2). The same experiment on adult keratinocytes from a female Asian donor aged 47 resulted in comparable results; an upregulation of 16.53fold ± 0.98SE and 4.90fold ± 0.17SE by SRM1650b and SRM2975, respectively, after 24h. Inhibition by 10 µM BDDI was 74.8% and 98.0%, respectively. Thus, SRM1650b resulted in a stronger upregulation of Cyp1A1 gene expression compared to SRM2975 on cells of both donors. Therefore the following experiments were performed only with SRM1650b.

SRM1650b enhanced POMC gene expression 1.5-fold ± 0.03SE significantly after 6h. Again BDDI was able to significantly protect against this induction at 10 µM and 2.5 µM (Fig. 3). The cytokine IL-6 gene was expressed 1.4fold ± 0.04SE elevated by SRM1650b after 6h. BDDI inhibited this upregulation significantly at 10 µM and 2.5 µM (Fig. 4).

Additionally SRM1650b significantly increased MMP-1 gene expression 2.4fold ± 0.05SE after 24h. BDDI lowered significantly at 1 µM the gene induction to 0.63fold ± 0.03SE versus untreated (Fig. 5).

CONCLUSION

These studies nicely showed that SRM1650b and SRM2975 as surrogates for authentic street particulate matter were able to stimulate AhR pathway in epidermal keratinocytes measured as upregulated Cyp1A1 expression. Induction of premature aging was measured by upregulation of POMC, IL-6 and MMP-1 gene expression after incubation with SRM1650b.

Application of newly identified AhR antagonist BDDI was able to prevent the upregulation of Cyp1A1 induced by DEPs (SRM1650b and SRM2975). Also the SRM1650b induced POMC, IL-6 and MMP-1 gene expression could be diminished by BDDI. Concluding, the cosmetic active E2/2-benzylindene-5,6-dimethoxy-3,3-dimethylindan-1-one (BDDI) is capable to protect against air pollution induced premature signs of aging comprising wrinkle formation, hyperpigmentation and inflamming processes.

REFERENCES

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9. NIST: Certificate of Analysis, SRM 1650a, Diesel Particulate Matter, Gaithersburg MD, 27 Sept 2006
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