Induction of AHR Gene Expression in Colorectal Cancer Cell Lines by Cucurbitacin D, E, and I

Younes Aftabi[1,3], Habib Zarredar[1], Mohammadreza Sheikhi[2], Zahra Khoshkam[2], Abasalt Hosseinzadeh Colagar[3*]

[1] Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Tehran, Tehran, Iran
[3] Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Mazandaran, Iran

Abstract

Aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor and its induction may result in suppressing of cell proliferation in colorectal cancer (CRC). Cucurbitacin D (CucD), E (CucE) and I (CucI) are plant derived metabolites that inhibit cancer cells. This study aimed to evaluate the possible potency of the cucurbitacins for activation of AHR expression in CRC cell lines SW-480 and HT-29. The MTT assay was used to find the IC50 value of the metabolites in the cell lines. Afterwards, the cells were incubated with the IC50 concentrations of the cucurbitacins and AHR-mRNA expression assessed using RT-PCR. The IC50 values of CucD, CucE, and CucI were 4.5, 6.8, and 3.8 μM in HT-29 cell line and 35, 19, 17.5 μM in SW-480 cells, respectively. The SW-480 cells were more resistant against cucurbitacins in comparison with HT-29 cells and all three cucurbitacins led to more AHR-mRNA expression in HT-29 cells. CucE had the lowest effect on AHR-mRNA expression in the cell lines and CucI was a common metabolite for both HT-29 and SW-480 cells, which showed the lowest IC50 value (the highest toxicity) and the highest effect on AHR-mRNA expression. CucI may have a potential AHR-induction role and it could be applicable as an AHR-expression inducer in CRC studies.

Keywords: Aryl hydrocarbon receptor, Colon cancer, Cucurbitacin, HT-29, SW-480

Introduction

It is predicted that the global burden of colorectal cancer (CRC) will increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030 (Arnold et al., 2017). The Aryl-hydrocarbon receptor (AHR) is a ligand-activated transcription factor that upon activation, translocates to the nucleus and associates with ARNT, binds to the cognate dioxin responsive elements (DRE) and transactivates target genes, particularly the phase I and II drug-metabolizing enzymes (Nebert et al., 2004; Kawajiri and Fujii-Kuriyama, 2007). It controls a wide range of developmental and toxicological processes (Stockinger et al., 2014; Liu et al., 2014; Esser and Rannug, 2015). Moreover, AHR gene communicates with several cellular signal transduction cascades to lead cell proliferation, cell cycle arrest, and apoptosis (Marlowe and Puga A, 2005).

Some studies suggest that AHR may act as a tumor suppressor and its induction has been proposed as a potential target for cancer treatment (Fan et al., 2010; Wang et al., 2017; Kolluri et al., 2017). It is reported that lung cancer cell migration is inhibited by AHR overexpression (Tsai et al., 2017) and ligand-activation of the AHR exhibits enhanced antitumor effects in colon cell lines (Megna et al., 2017). The AHR-mRNA expression level is reported to be moderate in normal colon tissue and it has been shown that AHR pathway is active in CRC cell lines (Li et al., 1998; Koliopanos et al., 2002). Although, AHR has a critical role in suppression of intestinal carcinogenesis (Kawajiri et al., 2009), the molecular features of this event is not clarified convincingly. However, it was revealed already that the sustained AHR activation results in G1 phase cell cycle arrest (Levine-Fridman et al., 2014).

AHR activation could be reached through several ways: 1- Toxic ligands (Morrow et al., 2014), 2- Rapidly metabolized or relatively non-toxic ligands (Koliopanos et al., 2002; Ehrlich and Kerkvliet, 2017), 3- Nontoxic ligands (Goettel et al., 2016) and 4- An indirect ligand-independent event (Maayah et al., 2013). However, most ligands of this protein have been disqualified for pharmaceutical development regarding their toxicity potentials (Ehrlich and Kerkvliet, 2017). But, ligand-independent induction of AHR has been reported as

---

a useful strategy in cancer cell suppressing (Gluschnaider et al., 2010). Therefore, identifying appropriate molecules, which perform such function, could help in developing more successful cancer suppressing drugs.

Cucurbitacins (Cucs) are diverse plant derived metabolites that have been introduced as candidates for cancer cell inhibition (Lee et al., 2010). Structurally, they are a multiplex category of triterpenes such as cucurbitacin D (CucD), E (CucE) and I (CucI) found in the members of Cucurbitaceae plants and several other families and possess immense pharmacological potential (Kaushik et al., 2015). The STAT3 and F-actin are the two main identified molecular targets of Cucs. They induce G2/M (CucD and CucI) and/or S-phase (CucD) cell cycle arrest and exhibit an effective inhibitory action on many cells, including CRC cell lines (Chen et al., 2012). Cucs are not ligands of AHR and therefore, if they could induce AHR activity indirectly, they would be useful chemicals in the study of cancer cell inhibition via AHR-ligand independent activation. This study aimed to evaluate the effects of CucD, CucE, and CucI on AHR-mRNA expression in human primary colorectal adenocarcinoma cell lines SW-480 and HT-29.

Materials and Methods

Chemicals and cell culture

All solvents and reagents used were purchased from Sigma (USA). The human cancer cell lines HT-29 and SW-480 were provided from Iranian Biological Resources Center's Cell Bank (Tehran, Iran). CucD, CucE, and CucI were obtained from Extrasynthese, Genay, France. HT-29 and SW-480 cells were cultured in the RPMI-1640 mix with sodium bicarbonate, streptomycin/penicillin, l-glutamine and 10% FBS. The cells were incubated at 37 °C in a water-saturated atmosphere of 5% CO_2 and 95% air until confluence. All reagents and medium were prepared just before use.

Cell viability assay and IC50 determining

Mortality of CRC cell lines SW-480 and HT-29 under CucD, CucE and CucI treatment was investigated by the colorimetric MTT assay (Edmondson et al., 1998). Cells were divided into a 96-well plate (15×10^3 cells/well for both cell lines) in the culture medium for 24 h. Next, they were treated with different concentrations of CucD, CucE and CucI (0.25, 1, 3, 5, 8, 12, 16, 30 and 50 μM for HT-29; 3, 7, 12, 18, 24, 28, 36, 40 and 50 μM for SW-480 cell line) in 0.1% (v/v) dimethyl sulfoxide with at least 3 repeats for 24 h. Blank was also

measured in the absence of cells. Cells in culture medium and DMSO (0.1%, v/v) in the absence of drugs were considered as controls. Every assay was repeated three times. For the colorimetric MTT assay, 20 μl MTT, a soluble tetrazolium salt solution, (5 mg/ml in PBS), was added to the wells containing 80 μl medium in the absence of drugs. Plates were incubated for 3 h at 37◦C in the dark. Cells were then solubilized by adding 100 μl of 0.04 N DMSO and formazan absorbance was recorded at 550 nm using a Microplate Reader RT2100C spectrophotometer (Rato Life and Analytical Sciences Co., China). Cell growth percentage was calculated as [mean of the test well (3 repeats) − mean of the blank wells] × 100/ [mean of control wells − mean of blank wells]. Plots of viable cells percentage against Cucs concentration series were drawn. The IC50 values (concentration of Cucs that decreases cell viability by 50%) were derived from the data plots using corresponding horizontal and vertical lines.

Assessing of the AHR-mRNA expression

SW-480 and HT-29 cells (5×10^5 cells per well) were seeded into 6-well plates and were grown to 80% confluency. 24 h after treatment with cucurbitacins D, E, and I at IC50 concentrations, cells were harvested and total RNA was extracted from the cells by RNX-Plus solution (Sinaclon Labware & Container, Iran) according to the manufacturer's instructions. The cDNA was synthesized using Easy cDNA Synthesis Kit (Cat. No. A101161, Parstous Biotechnology, Iran) according to the manufacturer's instructions. The real-time PCR experiments were performed at least in duplicate using a 48 well Step One Real-Time PCR System and Real Q Plus Master Mix Green kit (Ampliqon A/S, Denmark) with the following conditions: 95 °C for 15 min, 40 amplification cycles consisting of 95 °C for 15 sec, 60 °C for 30 sec, and 72 °C for 60 sec. Melting curves were then determined with temperature ranging from 60 to 95 °C. GAPDH was chosen as an internal control. SYBR Green reagents were used for all real time PCR reactions. The expression of the genes was analyzed based on the cycle threshold (Ct) and relative expression levels were determined as $2^{-[\Delta\Delta C(t)]}$. The specific primers were used for AHR (F: CCATCCCCATACCCCACTAC, R: TTCTGGCTGGCACTGATACA) and GAPDH (F: GACCCCTTCATTGACCTCAACTAC; R: TCGCTCCTGGAAGATGGTGATGG).

Statistical analysis

In order to make it possible to compare Cucs effect on AHR-mRNA expression, the quantity of

mRNA upregulation under a specific Cuc (folds of expression increasing) divided into the IC50 value of corresponding cucurbitacin and named Index A. Therefore, the index A implies the upregulation amount of AHR-mRNA per each concentration unit of a particular Cuc. Indeed, characteristic drug features are reflected in the gene expression profile (Iskar et al., 2010). One-way ANOVA test and LSD post hoc analysis was used to evaluate IC50 and AHR-mRNA expression data. The statistical significance level was set at $P<0.05$. SPSS version 20.0 was employed for the data analyzing.

Results

Cell viability and IC50

Figure 1A and Figure 2A depict the cell viability vs. gradually increasing concentration of the Cucs in HT-29 and SW-480 cell lines, respectively. The behavior of CucD, CucE, and CucI in two cell lines was different, but in the final stage, CucI showed more toxicity in both of them. The IC50 values of CucD, CucE, and CucI were 4.5, 6.8, and 3.8 μM in HT-29 cells (Figure 1B) and 35, 19, 17.5 μM in SW-480 cell line, respectively (Figure 2B). Comparison of IC50 values of CucE and CucI in SW-480 cells showed no significant difference but other comparisons result in significant differences (Table 1). However, in HT-29 cells the IC50 values of all Cucs were significantly different with each other (Table 1). In controls, which were treated with DMSO (0.1%, v/v) and medium, no significant change in cell viability was detected.

Table 1. Comparison of IC50s and AHR-mRNA expression in the cell lines after treating with Cucs

Cell line	Compared Cucs	P-value of IC50s comparison	P-value of index A* comparison
HT-29	D, E	0.000	0.000
	D, I	0.007	0.226
	E, I	0.000	0.000
SW-480	D, E	0.000	0.085
	D, I	0.000	0.000
	E, I	0.347	0.000

*Index A: Folds of AHR-mRNA expression increasing/IC50 concentration of Cucs.


(A)

(B)   (C)
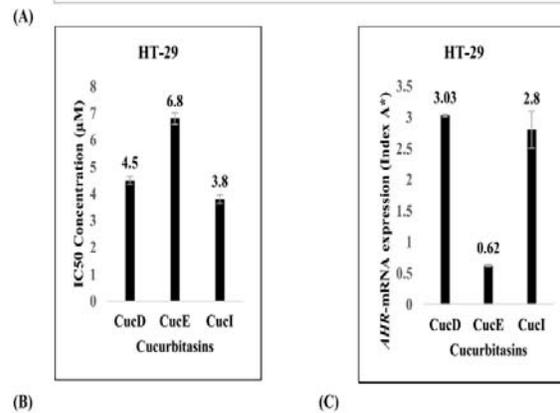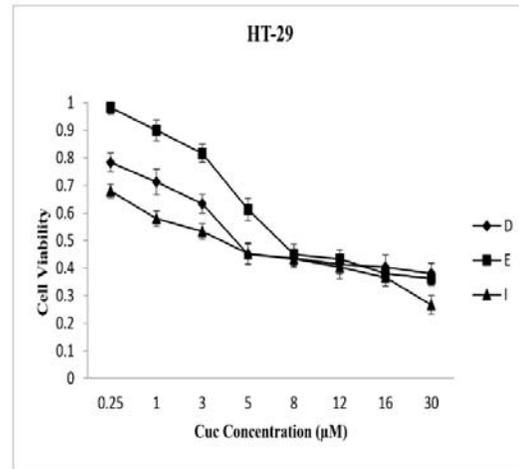
Figure 1. Cell viability, IC50 value and AHR-mRNA expression under CucD, CucE and CucI treatment in HT-29 cells. (A) The lethality of the gradually increasing concentration of CucD, CucE and CucI in HT-29 cell line. Results represented as a percentage of control recovery, which was considered to be 100%. All data were reported as the mean (±S.E.M.) of at least three separate experiments. (B) IC50 concentrations of cucurbitacins in HT-29 cell line. (C) AHR-mRNA expression after treatment with the cucurbitacins in HT-29 cells. *Index A: Folds of AHR-mRNA expression increasing/IC50 concentration of Cucs.

AHR-mRNA expression

Melting curves showed that the primers are efficient for gene expression analysis (Figure 3). AHR-mRNA expression upregulated in HT-29 cells after treatment with CucD, CucE, and CucI 13.63, 4.21 and 10.64 folds, respectively. Moreover, in SW-480 cells AHR-mRNA increased 3.5, 1.14 and 8.05 folds under CucD, CucE and CucI treatments, respectively. In HT-29 cells, index A was not significantly different for CucD, CucI comparison (Table 1). Also, in the SW-480 cell line difference between the index A of CucD and CucE was not statistically significant. All other comparisons of the

index A for Cucs showed a significant difference (Table 1).

(A)

(B)   **(C)**
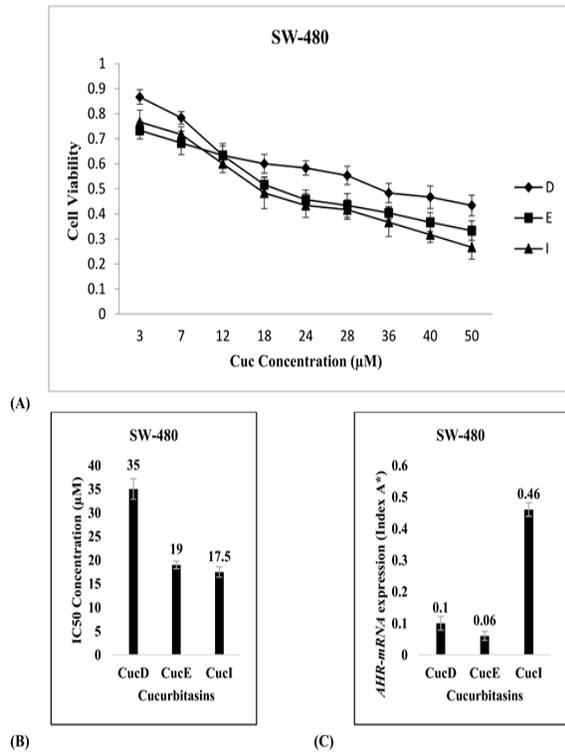
Figure 2. IC50 value and AHR-mRNA expression under CucD, CucE and CucI treatment in SW-480 cell lines. (A) The lethality of the gradually increasing concentration of CucD, CucE, and CucI in SW-480 cell line. Results represented as a percentage of control recovery, which was considered to be 100%. All data were reported as the mean (±S.E.M.) of at least three separate experiments. IC50 concentrations of cucurbitacins in SW-480 cell line. (B) IC50 concentrations of cucurbitacins in SW-480 cells. (C) AHR-mRNA expression after treatment with the cucurbitacins in SW-480 cell line. *Index A: Folds of AHR-mRNA expression increasing/IC50 concentration of Cucs.

Discussion

Colorectal cancer is the third most commonly diagnosed malignancy and the fourth leading cause of cancer-related deaths in the world. Its incidence and mortality rates are rising rapidly in many countries and the number of patients with CRC will continue to increase in future decades. Therefore, improvement of treatment options of CRC is a vital issue (Arnold et al., 2017).

The AHR gene contributes to cell proliferation, cell cycle arrest and apoptosis and therefore has a crucial role in cancer-related molecular pathways (Marlowe and Puga A, 2005; Fan et al., 2010). However, the role of AHR gene in cancer has remained controversial and recent evidence supports both pro- and anti-carcinogenic properties of AHR signaling (Xie and Raufman, 2015). Besides, the induction of tumor suppressor and anti-metastatic function of AHR has been proposed as a potential target for cancer treatment (Kolluri et al., 2017; Tsai et al., 2017; Denison and van den Berg, 2017). Recently, it is reported that AHR activation may induce p21cip1/waf1 and lead to anti-proliferative effects (O'Donnell et al., 2017). Especially, it is also shown recently that AHR may represent a potential putative target for novel anticancer agents for CRC (Megna et al., 2017). AHR pathway role in intestinal cancers has been subject of many molecular studies and its protective role in tumorigenesis has been emphasized (Kawajiri et al., 2009; Díaz-Díaz et al., 2014; Ikuta et al., 2016; Ikuta et al., 2013; Ronnekleiv-Kelly et al., 20126; Oh-oka et al., 2017). Altogether, Ikuta et al. concluded that in normal intestine tissue, AHR is associated with tumor prevention by regulating gut immunity, whereas in tumor cells, it is involved in growth suppression (Hall et al., 2010).

AHR could be activated by toxic ligands, rapidly metabolized or relatively non-toxic ligands, non-toxic ligands, and even ligand independently and then prevent cancer cell growth and migration (Koliopanos et al., 2002; Morrow et al., 2014; Ehrlich and Kerkvliet, 2017; Hall et al., 2010; Safe et al., 2010; Chen et al., 2001). However, given that many AHR agonists are potential oxidative stress inducers (Qiang et al., 2004; Abdelrahim et al., 2006), ligand-independent or nontoxic metabolites-dependent induction of AHR may have a priority for using as AHR inducers (Ehrlich and Kerkvliet, 2017). For instance, Megna et al. (2017) showed that piperidone analogues of curcumin, an AHR ligand, exhibit enhanced antitumor effects in colon cell lines due to the ability of these compounds in AHR activation. In the same way, Gluschnaider et al. indicated a ligand-independent strategy of boosting AHR expression as a means of suppressing prostate cancer (Gluschnaider et al., 2010). Indeed, there are compounds such as Sunitinib (Maayah et al., 2013) Omeprazole and Ketoconazole (Novotna et al., 2014; Jin et al., 2014), which facilitate AHR activity and induce AHR-dependent pathways, but they are not AHR ligands or represent a weak affinity (Platten et al., 2015).

Cucurbitacins are plant-derived highly oxygenated triterpenes that exhibit anti-cancer activity. Indeed, many plants, which have been used in folk medicine to treat cancer, contain these

metabolites (Lee et al., 2010; Alghasham, 2013; Platten et al., 2015; Jafargholizadeh et al., 2018). Among different Cucs, it is shown previously that CucD, CucE and CucI exhibit a potent inhibitory

concentration unit (Index A) of CucD in comparison with CucE showed no significant difference in HT-29 cells (Table1). Also, in SW-480 cell line CucE and CucI showed no significant difference in IC50
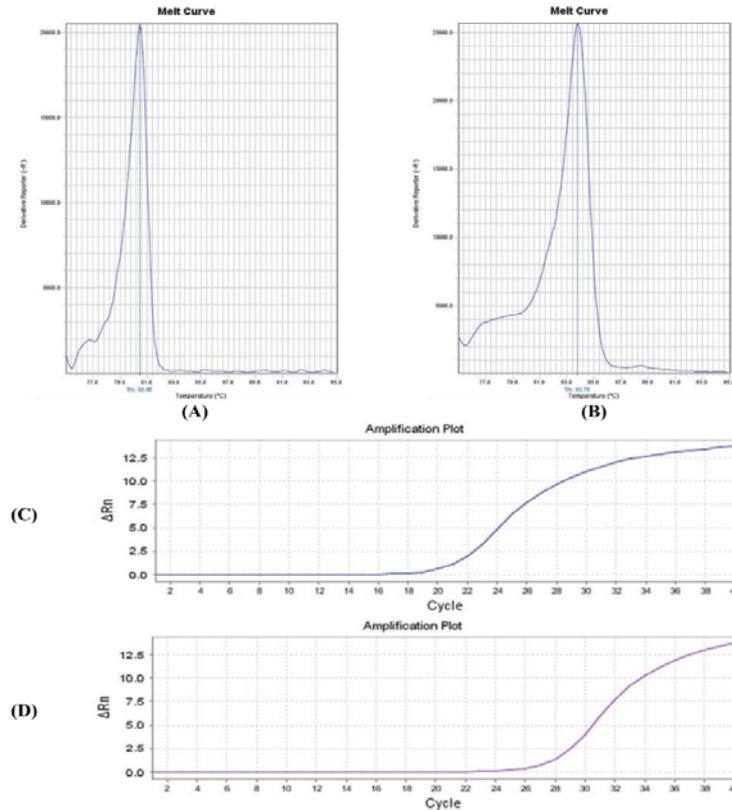
Figure 3. Melting Curves of GAPDH (A) and AHR (B). Amplication plots of GAPDH (C) and AHR (D).

effect on the CRC cells (Jayaprakasam et al., 2003). For instance, Hsu et al. (2014) showed that CRC primary cells accumulated in metaphase due to G2/M arrest after treating with 2.5-7.5 μM of CucE. Feng et al (2014) found that it inhibits SW-480 cells proliferation and modulates the expression of cell cycle regulators through Wnt/β-catenin signaling activation and upregulation of a tumor suppressor. Further, Kim et al. (2014) reported that up to 500 nM CucI reduces SW-480 cells proliferation by enhancing apoptosis and cell cycle arrest at the G2/M phase with a decreased expression of cell cycle proteins and increased caspases activities. Also, Song et al. (2015) demonstrated that CucI decreases the viability of COLO205 cell line significantly (IC50=200 nM/24h) and suppresses cell migration and invasion and decreases expression of p-STAT3 and MMP-9.

In the current study, the comparison of IC50 value between CucD and CucE; CucD and CucI; and CucE and CucI in HT-29 cells showed that there are significant differences between them (Table1). However, AHR-mRNA expression per

value, however, the AHR expression per concentration unit of CucE and CucI in this cell line was significantly different (Table1). Indeed, in SW-480 cells CucI causes to 7.67 folds more AHR-mRNA expression in comparison with CucE (Figure 2C). Regarding that alongside common effects, there are some different induced pathways by these two cucurbitacins (Chen et al., 2012), the difference of AHR-mRNA induction sounds reasonable.

The SW-480 cells were more resistant against Cucs in comparison with HT-29 cells. Indeed, in comparison with HT-29 cells, respectively 7.78, 2.8 and 4.6 folds more of CucD, CucE and CucI were needed to kill 50 percent of SW-480 cells. Also, all three Cucs lead to more AHR-mRNA expression in HT-29 cells. In other words, each concentration unit of CucD, CucE and CucI results in 33, 10.3 and 6.09 folds more AHR-mRNA expression in HT-29 cells in comparison with SW-480 cells.

In the HT-29 cells, the order of Cucs from the highest cytotoxicity to the lowest was: CucI>CucD>CucE (Figure 1B). However, the

71

cytotoxicity order of metabolites in SW-480 cell line was CucI>CucE>CucD without significant difference between CucI and CucE (Figure 2B, Table 1). In a similar manner, it is previously reported that compared to CucD, CucI and CucE represent a higher toxicity in SW-1353 cell line (Abbas et al., 2013). Index A comparison revealed that in HT-29 cells the order of metabolites in affecting AHR-mRNA expression from the highest to the lowest could be shown as CucD>CucI>CucE (Figure 1C) and there was no significant difference between CucD and CucI (Table 1). In SW-480 cells the order of metabolites regarding their effect on AHR-mRNA expression was: CucI>CucD>CucE, without significant difference between CucD and CucE (Figure 2C).

The CucE in HT-29 cells had the lowest cytotoxicity and lowest effect on AHR-mRNA expression (Figures 1B and C). CucI showed higher cytotoxicity and higher effect on AHR-mRNA expression in SW-480 cell line (Figures 2B and C). However, regarding that there is no significant difference between CucD and CucI in AHR-mRNA expression in HT-29 cells, it could be inferred that among studied three cucurbitacins the CucI is a common metabolite for both HT-29 and SW-480 cell lines that has the lowest IC50 value (the highest toxicity) and the highest effect on AHR-mRNA expression.

According to recent reports, it seems that Cucs effect and AHR pathway have overlapping features in cancerous cells. The IDO-AHR-IL6-STAT3 signaling loop maintains indoleamine-2,3-dioxygenase (IDO) expression in human cancers (Litzenburger et al., 2014). Induction of IDO and IDO-mediated tryptophan catabolism has been introduced as an important immunoregulatory mechanism, which depends on AHR expression (Nguyen et al., 2014). Initial studies showed that the CucI is a selective inhibitor of JAK/STAT3 activation and reduces the levels of activated STAT3 in human cancer cell lines (Blaskovich et al., 2003). Then, it could be hypothesized that after incubating cells with CucI and disruption of IDO-AHR-IL6-STAT3 signaling loop via STAT3 inhibiting, AHR expression may upregulate to compensate the reduction of the loop outcome, however, excessive amounts of AHR protein results in increased expression of DRE-containing genes and lead to growth inhibition and apoptosis (Nebert et al., 2000). Also, a recent study revealed that CucE modulates AHR signaling in CD4$^+$ T cells and stimulates Cyp1A expression, which is hallmark of AHR activation (Jevtić et al., 2016). Furthermore, the activation of AHR results in cyclin D inhibiting and promotes S-phase arrest (Marlowe and Puga A, 2005) in a same way as CucD performs (Chan et al., 2010).

In conclusion, our findings revealed that the cucurbitacin D, E and I show different lethal concentrations in colorectal cancer cell lines HT-29 and SW-480. Also, there was a different level of AHR-mRNA expression under treatment with these metabolites. In comparison with SW-480 cells, the HT-29 cells were more vulnerable against the cucurbitacins and this cell line represented more AHR-mRNA expression too. Cucurbitacin I was common metabolite that resulted in the highest toxicity and the highest AHR-mRNA expression in HT-29 and SW-480 cells. Therefore, it may potentially be useful as an indirect activator of AHR pathway in colorectal cancer cells.

Acknowledgements

Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Abbas S., Vincourt J. B., Habib L., Netter P., Greige-Gerges H. and Magdalou J. (2013) The cucurbitacins E, D and I: investigation of their cytotoxicity toward human chondrosarcoma SW 1353 cell line and their biotransformation in man liver. Toxicology letters 216:189-199.
2. Abdelrahim M., Ariazi E., Kim K., Khan S., Barhoumi R., Burghardt R., Liu S., Hill D., Finnell R., Wlodarczyk B. and Jordan, V. C. (2006) 3-Methylcholanthrene and other aryl hydrocarbon receptor agonists directly activate estrogen receptor α. Cancer research 66:2459-2467.
3. Alghasham A. A. (2013) Cucurbitacins–a promising target for cancer therapy. International journal of health sciences 7:77.
4. Arnold M., Sierra M. S., Laversanne M., Soerjomataram I., Jemal A. and Bray F. (2017) Global patterns and trends in colorectal cancer incidence and mortality. Gut 66:683-691.
5. Blaskovich M. A., Sun J., Cantor A., Turkson J., Jove R. and Sebti S. M. (2003) Discovery of JSI-124 (cucurbitacin I), a selective Janus kinase/signal transducer and activator of

transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice. Cancer research 63:1270-1279.

6. Chan K. T., Meng F. Y., Li Q., Ho C. Y., Lam T. S., To Y., Lee W. H., Li M., Chu K. H. and Toh M. (2010) Cucurbitacin B induces apoptosis and S phase cell cycle arrest in BEL-7402 human hepatocellular carcinoma cells and is effective via oral administration. Cancer letters 294:118-124.

7. Chen I., Hsieh T., Thomas T. and Safe, S. (2001) Identification of estrogen-induced genes downregulated by AhR agonists in MCF-7 breast cancer cells using suppression subtractive hybridization. Gene 262:207-214.

8. Chen X., Bao J., Guo J., Ding Q., Lu J., Huang M. and Wang Y. (2012) Biological activities and potential molecular targets of cucurbitacins: a focus on cancer. Anti-cancer drugs 23:777-787.

9. Denison M. S. and van den Berg M. (2017) Editorial overview: The aryl hydrocarbon (Ah) receptor: From toxicology to human health. Current opinion in toxicology 2:i-iv.

10. Díaz-Díaz C. J., Ronnekleiv-Kelly S. M., Nukaya M., Geiger P. G., Balbo S., Dator R., Megna B. W., Carney P. R., Bradfield C. A. and Kennedy G.D. (2016) The Aryl Hydrocarbon Receptor is a Repressor of Inflammation-associated Colorectal Tumorigenesis in Mouse. Annals of surgery 264:429-436.

11. Edmondson J. M., Armstrong L. S. and Martinez A. O. (1988) A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures. Journal of tissue culture methods 11:15-17.

12. Ehrlich A. K. and Kerkvliet N. I. (2017) Is chronic AhR activation by rapidly metabolized ligands safe for the treatment of immune-mediated diseases?. Current opinion in toxicology 2:72-78.

13. Esser C. and Rannug A. (2015) The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. Pharmacological reviews 67:259-279.

14. Fan Y., Boivin G. P., Knudsen E. S., Nebert D. W., Xia Y. and Puga A. (2010) The aryl hydrocarbon receptor functions as a tumor suppressor of liver carcinogenesis. Cancer research 70:212-220.

15. Feng H., Zang L., Zhao Z. X. and Kan Q. C. (2014) Cucurbitacin-E inhibits multiple cancer cells proliferation through attenuation of Wnt/β-catenin signaling. Cancer biotherapy and radiopharmaceuticals 29:210-214.

16. Gluschnaider U., Hidas G., Cojocaru G., Yutkin V., Ben-Neriah Y. and Pikarsky E. (2010) β-TrCP inhibition reduces prostate cancer cell growth via upregulation of the aryl hydrocarbon receptor. PloS one 5:e9060.

17. Goettel J. A., Gandhi R., Kenison J. E., Yeste A., Murugaiyan G., Sambanthamoorthy S., Griffith A. E., Patel B., Shouval D. S., Weiner H. L. and Snapper S.B. (2016) AHR activation is protective against colitis driven by T cells in humanized mice. Cell reports 17:1318-1329.

18. Hall J. M., Barhoover M. A., Kazmin D., McDonnell D. P., Greenlee W. F. and Thomas, R. S. (2010) Activation of the aryl-hydrocarbon receptor inhibits invasive and metastatic features of human breast cancer cells and promotes breast cancer cell differentiation. Molecular endocrinology 24:359-369.

19. Hsu Y. C., Huang T. Y. and Chen M. J. (2014) Therapeutic ROS targeting of GADD45γ in the induction of G2/M arrest in primary human colorectal cancer cell lines by cucurbitacin E. Cell death & disease 5:e1198.

20. Ikuta T., Kobayashi Y., Kitazawa M., Shiizaki K., Itano N., Noda T., Pettersson S., Poellinger L., Fujii-Kuriyama Y., Taniguchi S.I. and Kawajiri K. (2013) ASC-associated inflammation promotes cecal tumorigenesis in aryl hydrocarbon receptor-deficient mice. Carcinogenesis 34:1620-1627.

21. Ikuta T., Kurosumi M., Yatsuoka T. and Nishimura Y. (2016) Tissue distribution of aryl hydrocarbon receptor in the intestine: Implication of putative roles in tumor suppression. Experimental cell research 343:126-134.

22. Iskar M., Campillos M., Kuhn M., Jensen L. J., Van Noort V. and Bork, P. (2010) Drug-induced regulation of target expression. PLoS computational biology 6:e1000925.

23. Jafargholizadeh N., Zargar S. J. and Aftabi Y. (2018) The cucurbitacins D, E, and I from Ecballium elaterium (L.) upregulate the LC3 gene and induce cell-cycle arrest in human gastric cancer cell line AGS. Iranian journal of basic medical sciences 21:253.

24. Jayaprakasam B., Seeram N. P. and Nair M. G. (2003) Anticancer and antiinflammatory activities of cucurbitacins from Cucurbitaandreana. Cancer letters 189:11-16.

25. Jevtić B., Djedović N., Stanisavljević S., Despotović J., Miljković D. and Timotijević G. (2016) Cucurbitacin E potently modulates the activity of encephalitogenic cells. Journal of agricultural and food chemistry 64:4900-4907.

26. Jin U. H., Lee S. O., Pfent C. and Safe S. (2014) The aryl hydrocarbon receptor ligand omeprazole inhibits breast cancer cell invasion and metastasis. BMC cancer 14:498.

27. Kaushik U., Aeri V. and Mir S. R. (2015) Cucurbitacins–an insight into medicinal leads from nature. Pharmacognosy reviews 9:12.

28. Kawajiri K. and Fujii-Kuriyama Y. (2007) Cytochrome P450 gene regulation and physiological functions mediated by the aryl

73

hydrocarbon receptor. Archives of biochemistry and biophysics 464:207-212.

29. Kawajiri K., Kobayashi Y., Ohtake F., Ikuta T., Matsushima Y., Mimura J., Pettersson S., Pollenz R. S., Sakaki T., Hirokawa T. and Akiyama T. (2009) Aryl hydrocarbon receptor suppresses intestinal carcinogenesis in ApcMin/+ mice with natural ligands. Proceedings of the national academy of sciences 106:13481-13486.

30. Kim H. J., Park J. H. Y. and Kim J. K. (2014) Cucurbitacin-I, a natural cell-permeable triterpenoid isolated from Cucurbitaceae, exerts potent anticancer effect in colon cancer. Chemico-biological interactions 219:1-8.

31. Koliopanos A., Kleeff J., Xiao Y., Safe S., Zimmermann A., Büchler M. W. and Friess H. (2002) Increased arylhydrocarbon receptor expression offers a potential therapeutic target for pancreatic cancer. Oncogene 21:6059.

32. Kolluri S. K., Jin U. H. and Safe S. (2017) Role of the aryl hydrocarbon receptor in carcinogenesis and potential as an anti-cancer drug target. Archives of toxicology 91:2497-2513.

33. Lee D. H., Iwanski G. B. and Thoennissen N. H. (2010) Cucurbitacin: ancient compound shedding new light on cancer treatment. The scientific world journal 10:413-418.

34. Levine-Fridman A., Chen L. and Elferink C. J. (2004) Cytochrome P4501A1 promotes G1 phase cell cycle progression by controlling aryl hydrocarbon receptor activity. Molecular pharmacology 65:461-469.

35. Li W., Harper P. A., Tang B. K. and Okey A. B. (1998) Regulation of cytochrome P450 enzymes by aryl hydrocarbon receptor in human cells: CYP1A2 expression in the LS180 colon carcinoma cell line after treatment with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin or 3-methylcholanthrene. Biochemical pharmacology 56:599-612.

36. Litzenburger U. M., Opitz C. A., Sahm F., Rauschenbach K. J., Trump S., Winter M., Ott M., Ochs K., Lutz C., Liu X. and Anastasov N. (2014) Constitutive IDO expression in human cancer is sustained by an autocrine signaling loop involving IL-6, STAT3 and the AHR. Oncotarget 5:1038.

37. Liu H., Ramachandran I. and Gabrilovich D. I. (2014) Regulation of plasmacytoid dendritic cell development in mice by aryl hydrocarbon receptor. Immunology and cell biology 92:200-203.

38. Maayah Z. H., El-Gendy M. A., El-Kadi A. O. and Korashy H. M. (2013) Sunitinib, a tyrosine kinase inhibitor, induces cytochrome P450 1A1 gene in human breast cancer MCF7 cells through ligand-independent aryl hydrocarbon receptor activation. Archives of toxicology 87:847-856.

39. Marlowe J. L. and Puga A. (2005) Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. Journal of cellular biochemistry 96:1174-1184.

40. Megna B. W., Carney P. R., Depke M. G., Nukaya M., McNally J., Larsen L., Rosengren R. J. and Kennedy G.D. (2017) The aryl hydrocarbon receptor as an antitumor target of synthetic curcuminoids in colorectal cancer. Journal of surgical research 213:16-24.

41. Morrow D., Qin C., Smith III, R. and Safe, S. (2004) Aryl hydrocarbon receptor-mediated inhibition of LNCaP prostate cancer cell growth and hormone-induced transactivation. The Journal of steroid biochemistry and molecular biology 88:27-36.

42. Nebert D. W., Dalton T. P., Okey A. B. and Gonzalez F. J. (2004) Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. Journal of biological chemistry 279:23847-23850.

43. Nebert D. W., Roe A. L., Dieter M. Z., Solis W. A., Yang Y. I. and Dalton T. P. (2000) Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. Biochemical pharmacology 59:65-85.

44. Nguyen N. T., Nakahama T., Le D. H., Van Son L., Chu H. H. and Kishimoto T. (2014) Aryl hydrocarbon receptor and kynurenine: recent advances in autoimmune disease research. Frontiers in immunology 5:551.

45. Novotna A., Korhonova M., Bartonkova I., Soshilov A. A., Denison M. S., Bogdanova K., Kolar M., Bednar P. and Dvorak Z. (2014) Enantiospecific effects of ketoconazole on aryl hydrocarbon receptor. Plos one 9: e101832.

46. O'Donnell E. F., Jang H. S., Pearce M., Kerkvliet N. I. and Kolluri S. K. (2017) The aryl hydrocarbon receptor is required for induction of p21cip1/waf1 expression and growth inhibition by SU5416 in hepatoma cells. Oncotarget 8:25211.

47. Oh-oka K., Kojima Y., Uchida K., Yoda K., Ishimaru K., Nakajima S., Hemmi J., Kano H., Fujii-Kuriyama Y., Katoh R. and Ito, H. (2017) Induction of colonic regulatory T cells by mesalamine by activating the aryl hydrocarbon receptor. Cellular and molecular gastroenterology and hepatology 4:135-151.

48. Platten M., von Knebel Doeberitz N., Oezen I., Wick W. and Ochs, K. (2015) Cancer immunotherapy by targeting IDO1/TDO and their downstream effectors. Frontiers in immunology 5:673.

49. Qiang M. A., Kinneer K., Yongyi B. I. and KAN, Y. W. (2004) Induction of murine NAD (P) H: quinone oxidoreductase by 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin requires the CNC (cap n collar) basic leucine zipper transcription

74

factor Nrf2 (nuclear factor erythroid 2-related factor 2): cross-interaction between AhR (aryl hydrocarbon receptor) and Nrf2 signal transduction. Biochemical journal 377:205-213.

50. Ronnekleiv-Kelly S. M., Nukaya M., Díaz-Díaz C. J., Megna B. W., Carney P. R., Geiger P. G. and Kennedy G. D. (2016) Aryl hydrocarbon receptor-dependent apoptotic cell death induced by the flavonoid chrysin in human colorectal cancer cells. Cancer letters 370:91-99.

51. Safe S., Wormke M. and Samudio, I. (2000) Mechanisms of inhibitory aryl hydrocarbon receptor-estrogen receptor crosstalk in human breast cancer cells. Journal of mammary gland biology and neoplasia 5:295-306.

52. Song J., Liu H., Li Z., Yang C. and Wang C. (2015) Cucurbitacin I inhibits cell migration and invasion and enhances chemosensitivity in colon cancer. Oncology reports 33:1867-1871.

53. Stockinger B., Meglio P. D., Gialitakis M. and Duarte J. H. (2014) The aryl hydrocarbon receptor: multitasking in the immune system. Annual review of immunology 32:403-432.

54. Tsai C. H., Li C. H., Cheng Y. W., Lee C. C., Liao P. L., Lin C. H., Huang S. H. and Kang J.J. (2017) The inhibition of lung cancer cell migration by AhR-regulated autophagy. Scientific reports 7:41927.

55. Wang Z., Monti S. and Sherr D. H. (2017) The diverse and important contributions of the AHR to cancer and cancer immunity. Current opinion in toxicology 2:93-102.

56. Xie G. and Raufman J. P. (2015) Role of the aryl hydrocarbon receptor in colon neoplasia. Cancers 7:1436-1446.