

Quaternary benzo[c]phenanthridine alkaloids sanguinarine and chelerythrine do not affect transcriptional activity of aryl hydrocarbon receptor: Analyses in rat hepatoma cell line H4IIE.luc

Zdeněk Dvořák^{a,*}, Iva Sovadinová^b, Luděk Bláha^b, John P. Giesy^c, Jitka Ulrichová^a

^a Institute of Medical Chemistry and Biochemistry, Faculty of Medicine, Palacký University, Hněvotínská 3, 775 15 Olomouc, Czech Republic

^b Masaryk University Brno, Research Centre for Environmental Chemistry and Ecotoxicology (RECETOX), Kamenice 3, 625 00 Brno, Czech Republic

^c Michigan State University, 218c National Food Safety and Toxicology Center, East Lansing, MI 48824, USA

Received 19 August 2005; accepted 27 April 2006

Abstract

Quaternary benzo[c]phenanthridine alkaloids (QBAs) sanguinarine and chelerythrine exert a plethora of biological activities. Nevertheless, the specific cellular target for these alkaloids within the cell was not identified as far. Several literary data indicate that biological effects of QBAs could be associated with aryl hydrocarbon receptor (AhR) signaling pathway, including cytochrome P450 CYP1A, however, available information are controversial. In this work we analyzed the effects of sanguinarine and chelerythrine on AhR activity in rat hepatoma cells H4IIE.luc stably transfected with dioxin responsive element fused to luciferase gene (DRE-LUC). Studied QBAs were tested in submicromolar concentration range (0.0001–1 μM) and in incubation times 6, 24 and 48 h. Transcriptional activity of AhR was monitored by chemiluminescence measurement of luciferase catalytic activity. Sanguinarine and chelerythrine did not activate AhR in any time or dose tested. Chelerythrine (1 μM) but not sanguinarine caused moderate inhibition of AhR activation by 10 picomolar dioxin (exponential phase of receptor activation). In contrast, AhR activation by 2.5 nM dioxin (saturated receptor) was not affected by either alkaloid tested. In conclusion, the findings presented here favor rather for inactivity or modest inhibitory effect of QBAs on AhR signaling pathways *in vitro* than for the activation of the receptor. Regarding the concentrations of QBAs occurring *in vivo*, the use of products containing sanguinarine and/or chelerythrine has low toxicological potential in terms of the interactions with AhR signaling pathways.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Aryl hydrocarbon receptor; Transcription; Sanguinarine; Chelerythrine; Safety

1. Introduction

Quaternary benzo[c]phenanthridine alkaloids (QBAs) sanguinarine and chelerythrine (see Fig. 1) exert a wide

Abbreviations: AhR, aryl hydrocarbon receptor; BNF, beta-naphthoflavone; DMSO, dimethylsulfoxide; DRE-LUC, dioxin responsive element fused to luciferase reporter gene; FCS, foetal calf serum; CHE, chelerythrine; HepG2, human hepatoma cells; 3-MC, 3-methylcholanthrene; PAHs, polycyclic aromatic hydrocarbons; PKC, protein kinase C; QBA, quaternary benzo[c]phenanthridine alkaloid; SA, sanguinarine; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

* Corresponding author. Tel.: +420 58 5632324; fax: +420 58 5632302.

E-mail address: moulin@email.cz (Z. Dvořák).

array of biological activities, among which antimicrobial, antifungal and anti-inflammatory activities are the most important for practical applications of these compounds (Walterova et al., 1995). Two principal areas of QBAs practical use are dental care products (Giuliana et al., 1997; Hong et al., 2005), and feed additives in pig raising (Kosina et al., 2004). QBAs have multiple cellular targets that are determined in particular by: (i) reaction with nucleophilic and anionic moieties of amino acids in peptides and proteins (Schmeller et al., 1997); (ii) formation of molecular complex with DNA by intercalation (Maiti et al., 1982); (iii) inhibition of protein kinase C, Ca²⁺-dependent protein kinase C, cyclic AMP-dependent protein kinase, and

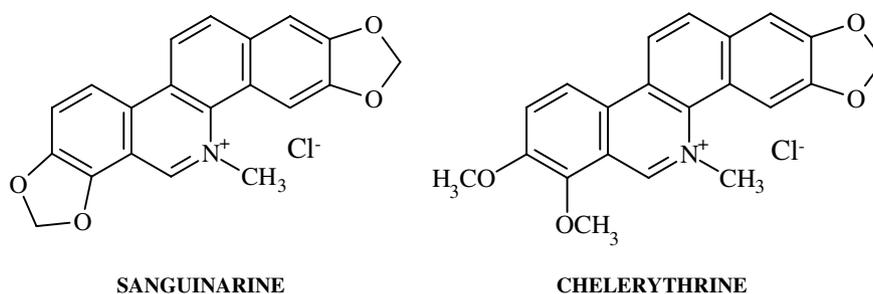


Fig. 1. Structures of sanguinarine and chelerythrine.

phospholipid-dependent protein kinase C (Wang et al., 1997).

There is increasing body of evidence that biological activities of QBAs could be linked to aryl hydrocarbon receptor (AhR) signaling pathway and cytochrome P450 CYP1A. The first indication that sanguinarine effects *in vivo* could be associated with AhR and/or CYP1A came from the observation, that sanguinarine toxicity in mice was attenuated by pre-treatment of the animals with CYP1A inducer 3-methylcholanthrene (Williams et al., 2000). Similar phenomenon was observed *in vitro*, when the cytotoxicity of sanguinarine in primary cultures of rat hepatocytes and in human hepatoma cells (HepG2) was significantly reduced by pre-treatment of the cells with CYP1A inducers dioxin, beta-naphthoflavone, and 3-methylcholanthrene (Vrba et al., 2004). There are also indications that CYP1A enzymes could be involved in the genotoxicity of sanguinarine and chelerythrine. The production of reactive species that formed DNA adducts was detected after the incubation of QBAs with rat liver microsomes isolated from the animals treated with CYP1A inducers (Stiborova et al., 2002). Hence, *in vivo* and *in vitro* toxicity of sanguinarine could be modulated by AhR-dependent signaling pathway. Another set of papers dealt with the effects of QBAs on AhR receptor. It was described that sanguinarine activates polycyclic aromatic hydrocarbon associated metabolic and signaling pathways in oral keratinocytes and tissues (Karp et al., 2005). The authors demonstrated that sanguinarine induces AhR-dependent expression of CYP1A and other genes. Nevertheless, the reliability of these results was impeached (Dvorak et al., 2005). In contrast, we have recently reported that sanguinarine and chelerythrine do not affect basal and dioxin-inducible AhR-dependent expression of CYP1A1 mRNA and protein in HepG2 cells (Zdarilova et al., 2006). In addition, both alkaloids inhibited catalytic activity of CYP1A1 (Karp et al., 2005; Vrba et al., 2004; Zdarilova et al., 2006). We concluded that CYP1A modulates QBAs cytotoxicity and genotoxicity, but QBAs themselves do not affect CYP1A1 expression (Zdarilova et al., 2006). In present work, as well as in our previous report, the submicromolar doses of QBAs were applied on the cells (Zdarilova et al., 2006). In higher concentrations (approx. 4 μ M), the inhib-

itory effects of QBAs against protein kinase C (PKC) must be taken into the consideration. Since functional PKC is essential for proper activation of AhR, the application of high concentrations of QBAs on the cells will logically result in the inhibition of dioxin-dependent AhR activation (Long et al., 1998). The question of effective inhibitory concentration of QBAs and their intrinsic cytotoxicity is often the subject of scientific disputations. Collectively, the literary data on the effects of QBAs on AhR transcriptional activity are ambiguous and further analyses are necessary.

The aim of our work was to determine, whether sanguinarine and chelerythrine in sub-micromolar concentrations activate AhR and whether these compounds interfere with dioxin-dependent AhR activation. Rat hepatoma cells H4IIE-*luc* cells stably transfected with dioxin-responsive elements fused to luciferase gene (DRE-LUC) were the model of choice. Transcriptional activity of AhR was monitored as luciferase catalytic activity.

2. Materials and methods

2.1. Chemicals

Dulbecco's modified Eagle's medium, minimal essential medium, foetal calf serum, streptomycin, penicillin, L-glutamine, non-essential amino acids and sodium pyruvate were purchased from Sigma Chemicals (St. Louis, MO). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) from Ultra Scientific (RI, USA). Sanguinarine and chelerythrine were isolated from sanguinrine (CAMAS Technologies, Inc., Broomfield, USA) using column chromatography on alumina (Dostal et al., 1992). Sanguinarine (SA) in 98.1% purity, M.P. 279–282 °C (Southon and Buckingham, 1989) and chelerythrine (CHE) in 95% purity, M.P. 200–204 °C (Southon and Buckingham, 1989) were obtained. All other chemicals were of the highest grade commercially available.

2.2. Cell cultures

The rat hepatoma cells H4IIE-*luc*, were stably transfected with a luciferase reporter gene fused to the dioxin-responsive elements (pGduLuc 1.1 reporter) as described elsewhere (Hilscherova et al., 2000; Sanderson et al., 1996). pGudLuc 1.1 reporter contains MMTV (mouse mammary tumor virus promoter) fragment as well as 480-bp fragment isolated from the 5' flanking region of the mouse Cyp1a1 gene (Sanderson et al., 1996). The 480-bp fragment contains four DREs. The cells were cultured in minimal essential medium supplemented with 10% heat-inactivated fetal calf serum. Cells were maintained at 37 °C in 5% CO₂ humidified incubator.

2.3. Cytotoxicity assays

H4IIE.luc cells were seeded on 24-well dishes at a density of 1×10^5 cells/well using culture medium enriched with foetal calf serum (10% v/v). Following 16 h of stabilization, the cells were treated for 6, 24 and 48 h with sanguinarine and chelerythrine in concentrations of 0.01–1 μ M or with dimethylsulfoxide (DMSO) as vehicle for control. Neutral red (NR) incorporation to the cells was measured as an indicator of cell viability. The concentrations of sanguinarine and chelerythrine that caused no more than a 20% decrease in cell viability were considered as non-toxic for further experiments.

2.4. AhR-dependent luciferase activity in H4IIE-luc cells

Rat hepatoma cells stably transfected with a luciferase reporter gene fused to the dioxin-responsive enhancers (H4IIE-luc) were seeded on 96-well culture plates and grown to 90–100% confluence. The cells were treated 6, 24 and 48 h with sanguinarine (SA; 0.0001 μ M, 0.001 μ M, 0.01 μ M, 0.1 μ M, 1 μ M), chelerythrine (CHE; 0.0001 μ M, 0.001 μ M, 0.01 μ M, 0.1 μ M, 1 μ M), and/or DMSO as vehicle for control in the presence or absence of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; final concentration 10 pM and/or 2.5 nM). The luciferase activity was measured on a luminometer TECAN GENios using the Luciferase Monitoring

Kit (Promega). The data were normalized per total protein content determined by bisinchoninic acid method (Stoscheck, 1990).

3. Results

3.1. Cytotoxicity assessment

Numerous studies employ the concentrations of QBAs that are without any doubt cytotoxic for the cells. In our experiments we considered concentrations of sanguinarine and chelerythrine that caused no more than a 20% decrease in cell viability as non-cytotoxic. Viability of the cells was assessed by incorporation of Neutral Red to H4IIE.luc cells. Rat hepatoma cells were incubated with tested compounds in concentration range 0.01–1 μ M for the periods of 6, 24 and 48 h. In parallel, the incubations with 1% v/v Triton X-100 or with dimethylsulfoxide (DMSO) as vehicle were performed to assess maximal and minimal cell damage, respectively. Both QBAs tested, i.e. sanguinarine and chelerythrine caused dose- and time-dependent damage of

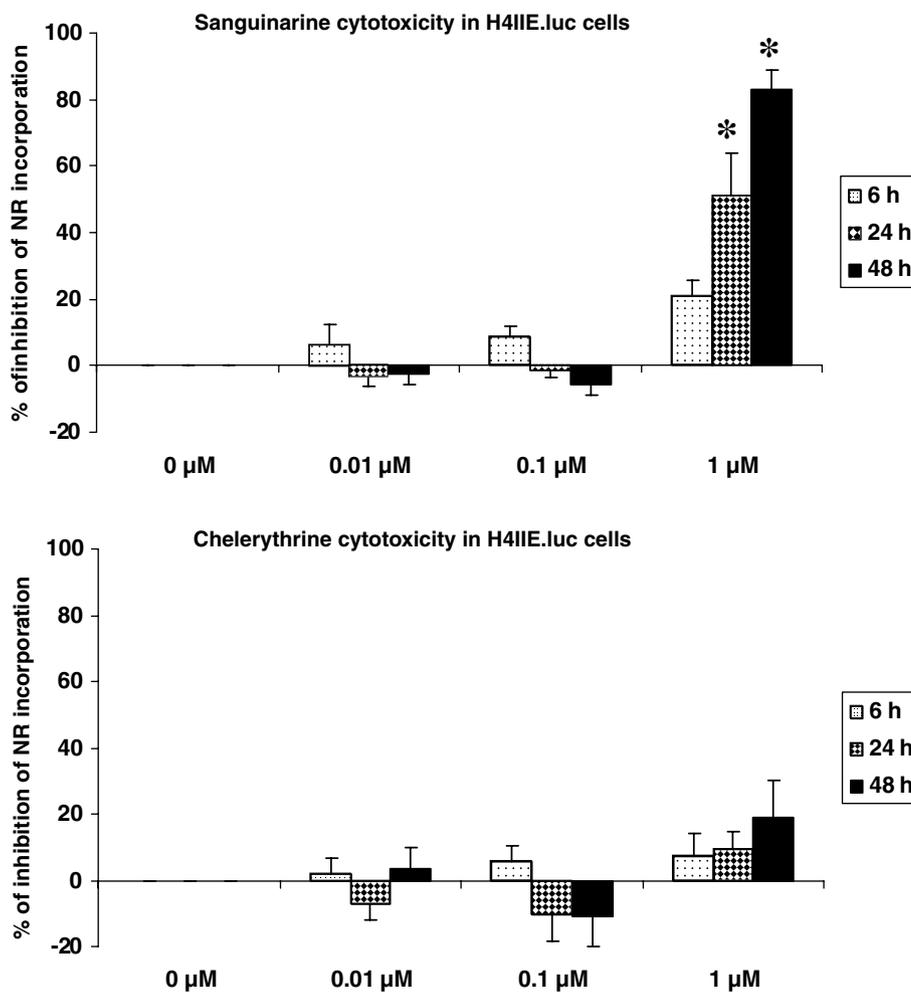


Fig. 2. Evaluation of sanguinarine and chelerythrine cytotoxicity in H4IIE.luc cells. Rat hepatoma cells were treated with tested compounds in concentration range 0.01–1 μ M and/or dimethylsulfoxide (DMSO) as vehicle for the periods of 6, 24 and 48 h. Cytotoxicity was assessed by monitoring incorporation of Neutral Red to the cells. Bar plots represent means \pm SD from three independent experiments. * = the value is significantly different from DMSO value at $p < 0.05$.

hepatoma cells. Cytotoxicity of chelerythrine was much lower than that of sanguinarine. Overall, we can consider chelerythrine as “non-toxic” in all tested concentrations and times of incubation. On the other hand, sanguinarine in 1 μM concentration was cytotoxic after 24 and 48 h of incubation. Dose- and time-effects of sanguinarine and chelerythrine on the viability of H4IIE.luc cells are shown in Fig. 2.

3.2. Effects of sanguinarine and chelerythrine on basal AhR activity in H4IIE.luc cells

In order to determine whether sanguinarine and chelerythrine activate AhR or not, we analyzed their effects in rat hepatoma cells stably transfected with dioxin-responsive elements fused to luciferase gene (H4IIE.luc cells), the experimental model routinely used for the screening on AhR activators (Hilscherova et al., 2000). Cells were treated

6, 24 and 48 h with tested QBAs in concentrations 0.0001–1 μM . Neither sanguinarine nor chelerythrine induced AhR-dependent luciferase activity in any concentration and time periods used (Fig. 3). These data imply that sanguinarine and chelerythrine are not the activators of AhR.

3.3. Time-course and dose–response effects of TCDD on AhR-dependent luciferase activity in H4IIE.luc cells

We challenged rat hepatoma cells for the periods of 6, 24 and 48 h with two different concentrations of TCDD (10 pM and/ 2.5 nM), the concentrations representing exponential phase of AhR activation and saturated AhR, respectively. Treatment of H4IIE.luc cells with TCDD in both concentrations caused a growing induction of AhR-dependent luciferase activity, the effect of 2.5 nM TCDD being more pronounced (Fig. 4A). The average fold

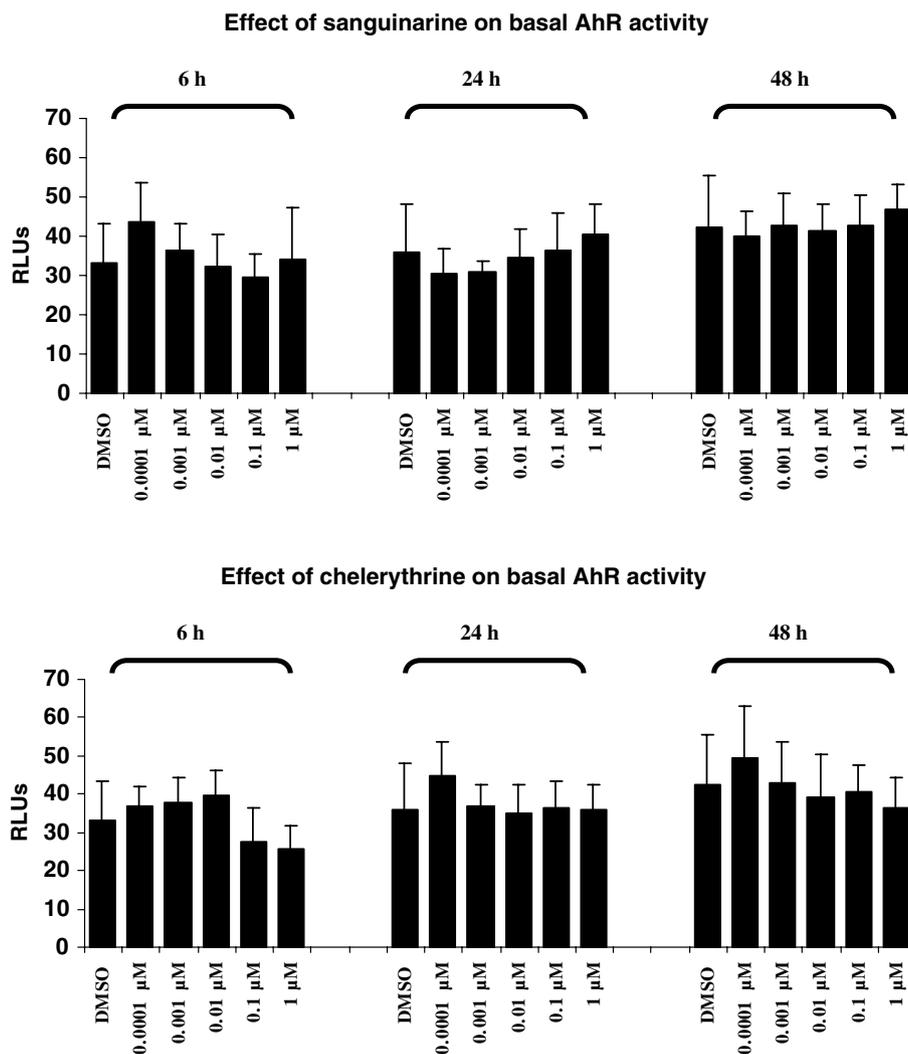


Fig. 3. Effects of sanguinarine and chelerythrine on basal activity of AhR in H4IIE.luc cell line. Cells were treated 6, 24 and 48 h with sanguinarine (SA; final concentrations 0.0001 μM , 0.001 μM , 0.01 μM , 0.1 μM , 1 μM), chelerythrine (CHE; final concentrations 0.0001 μM , 0.001 μM , 0.01 μM , 0.1 μM , 1 μM) and/or DMSO as vehicle for control. Luciferase activity was measured by chemiluminescence as described in Section 2. Bar graphs represent means \pm SD from three independent experiments. Within each experiment, four parallel measurements were acquired.

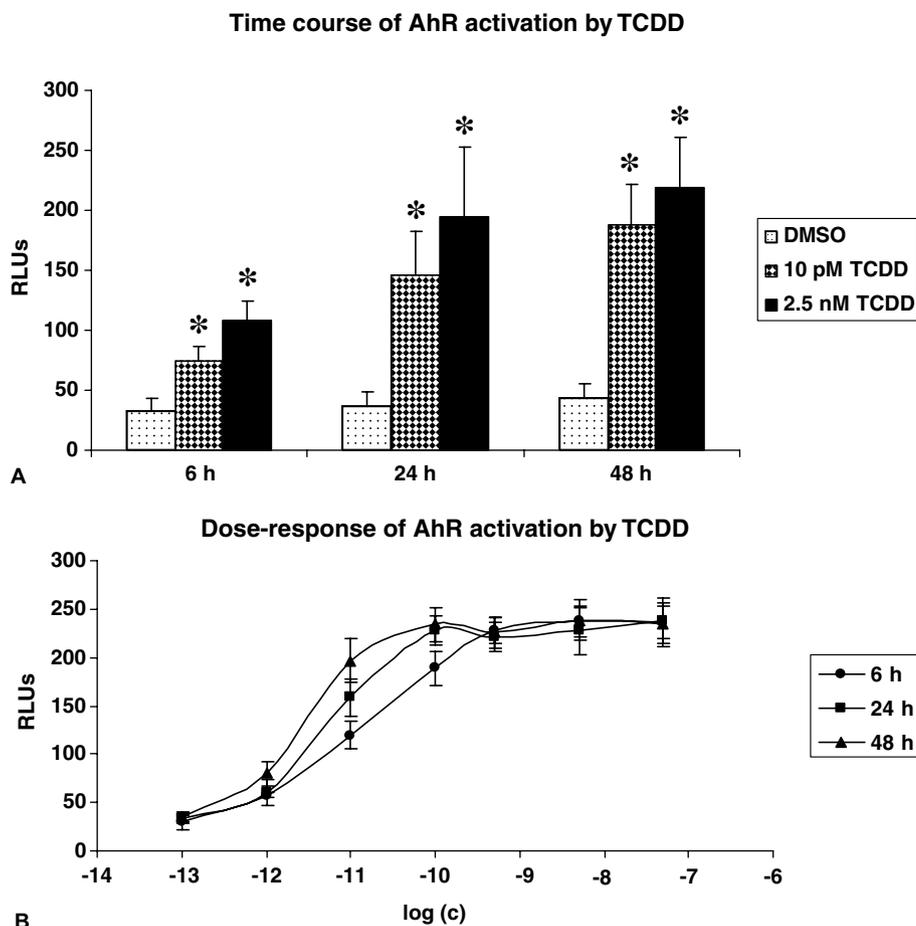


Fig. 4. Effect of dioxin on AhR-dependent luciferase activity in H4IE.luc cells. AhR activity was monitored as catalytic activity of luciferase by chemiluminescence as described in Section 2. Panel A: Time-course – cells were treated for 6, 24 and 48 h with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; final concentration 10 pM and 2.5 nM) and/or DMSO as a vehicle for control. Bar graphs represent the means \pm SD from three independent experiments. * – the value significantly different from the control value (DMSO) at $p < 0.05$. Panel B: Dose-response curves – cells were treated for 6, 24 and 48 h with TCDD in final concentrations from 1×10^{-13} M to 5×10^{-8} M. The plots represent the means \pm SD from three independent experiments.

induction of luciferase activity by 10 pM/2.5 nM TCDD was $2.2 \pm 0.4/3.3 \pm 0.5$ (after 6 h of the incubation); $4.1 \pm 1.0/5.4 \pm 1.6$ (after 24 h of the incubation) and $4.4 \pm 0.8/5.1 \pm 1.0$ (after 48 h of the incubation), respectively. In dose-response experiments, rat hepatoma cells were treated for 6, 24 and 48 h with TCDD in final concentrations ranging from 1×10^{-13} M to 5×10^{-8} M. Dose-response effects of TCDD on AhR-dependent luciferase activity show typical sigmoid profile (Fig. 4B).

3.4. Effects of sanguinarine and chelerythrine on AhR activated by TCDD

In the next series of experiments we analyzed the potential capability of sanguinarine and chelerythrine to inhibit dioxin-dependent AhR activation. Cells were treated 6, 24 and 48 h with TCDD (10 pM and/or 2.5 nM) in the presence of tested alkaloids (0.0001–1 μ M) and/or DMSO as vehicle for control. Sanguinarine did not affect AhR activation by both 10 pM and 2.5 nM TCDD in any time period tested (Figs. 5 and 6). Significant decrease of luciferase

activity after 24 and 48 h of treatment with 1 μ M sanguinarine (Figs. 5 and 6) is rather manifestation of sanguinarine cytotoxicity (Fig. 2) than the inhibition of AhR activity by this alkaloid. Chelerythrine caused moderate inhibition of TCDD-dependent AhR activity in H4IE.luc cells treated with 10 pM TCDD, however, the inhibition was statistically significant only for 1 μ M chelerythrine and 6 h of incubation. In contrast, the activation of AhR by 2.5 nM TCDD was not affected by chelerythrine in any concentration and time tested. Collectively, sanguinarine and chelerythrine in submicromolar concentrations do not inhibit dioxin-dependent AhR transcriptional activity in rat hepatoma cells.

4. Discussion

In this work we bring the evidence that quaternary benzo[*c*]phenanthridine alkaloids (QBAs) sanguinarine and chelerythrine in submicromolar concentrations do not affect transcriptional activity of aryl hydrocarbon receptor (AhR) in rat hepatoma cells H4IE.luc stably

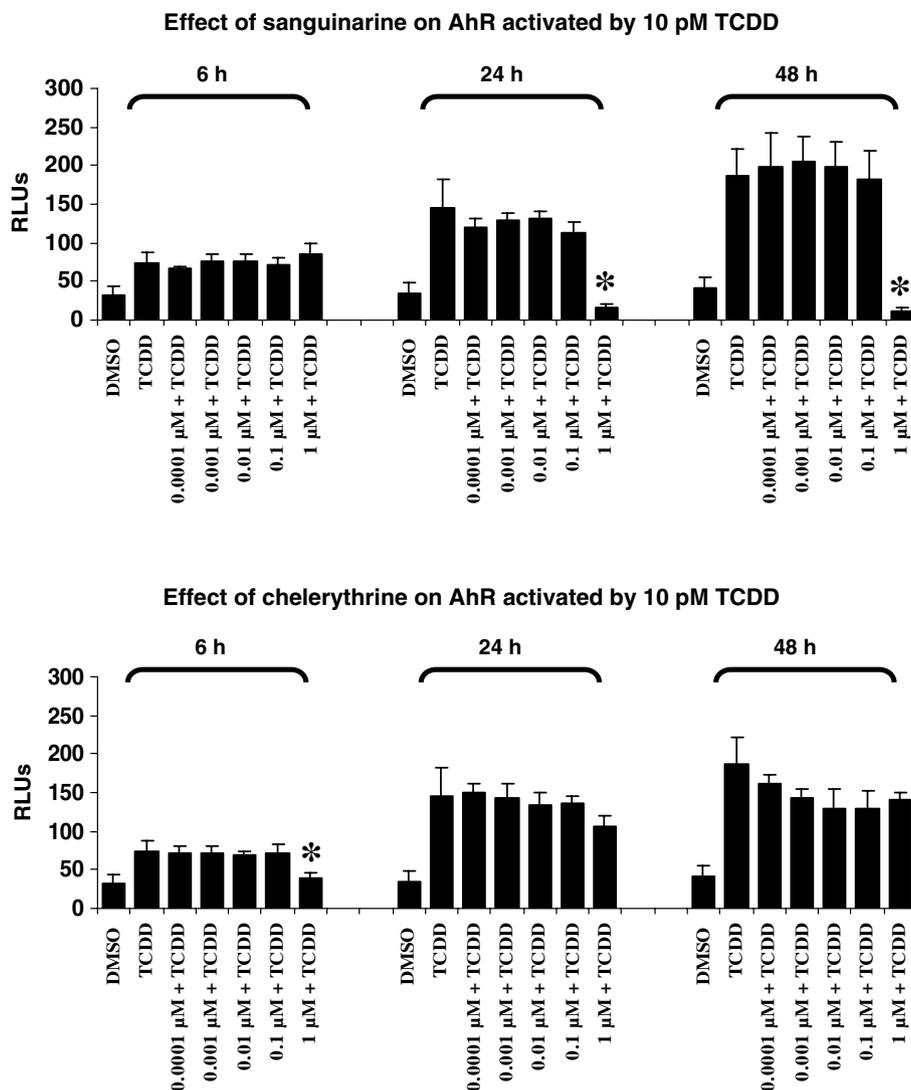


Fig. 5. Effects of sanguinarine and chelerythrine on AhR activated by 10 pM TCDD in H4IIE.luc cells. Cells were treated 6, 24 and 48 h with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; final concentration 10 pM) in the presence of sanguinarine (SA; final concentrations 0.0001 μM, 0.001 μM, 0.01 μM, 0.1 μM, 1 μM), chelerythrine (CHE; final concentrations 0.0001 μM, 0.001 μM, 0.01 μM, 0.1 μM, 1 μM) and/or DMSO as vehicle for control. Luciferase activity was measured by chemiluminescence as described in Section 2. Bar graphs represent means \pm SD from three independent experiments. Within each experiment, four parallel measurements were acquired. * – the value significantly different from the TCDD value at $p < 0.05$.

transfected with dioxin-responsive elements fused to luciferase reporter. This is supported by the findings that: (i) AhR-driven luciferase activity was not increased by QBAs; (ii) TCDD-induced luciferase catalytic activity was not altered by the presence of QBAs.

Putative interactions between QBAs and AhR received an attention in recent years. There are indications that AhR and/or CYP1A modulate cytotoxicity of sanguinarine both *in vitro* (Vrba et al., 2004) and *in vivo* (Williams et al., 2000). AhR-dependent signaling pathways play a role in modulation of sanguinarine genotoxicity as well (Stiborova et al., 2002). The findings of these papers are that activation of AhR by various ligands attenuates cytotoxicity and augments genotoxicity of sanguinarine. However, the mechanism was not elucidated yet. The role of CYP1A enzymes in this process is not clear and should be further

studied. Surprisingly, the approach of specific inhibition of CYP1A1/1A2 was not employed in any of the papers mentioned above. The phenomenon of the modulation of QBAs toxicity by AhR activation is attractive issue for molecular biologists and molecular toxicologists from mechanistic point of view. On the other hand, studying the effects of QBAs on AhR transcriptional activity is highly important in terms of the safety assessment for food, cosmetic and medical products containing QBAs. Interestingly, despite of the AhR importance in many cellular events, comprehensive study of the effects of QBAs on AhR-signaling pathways was not performed until this year. Recently it was reported that sanguinarine activates polycyclic aromatic hydrocarbon associated metabolic pathways in human oral keratinocytes and tissues (Karp et al., 2005). However, these data were the subject of dispu-

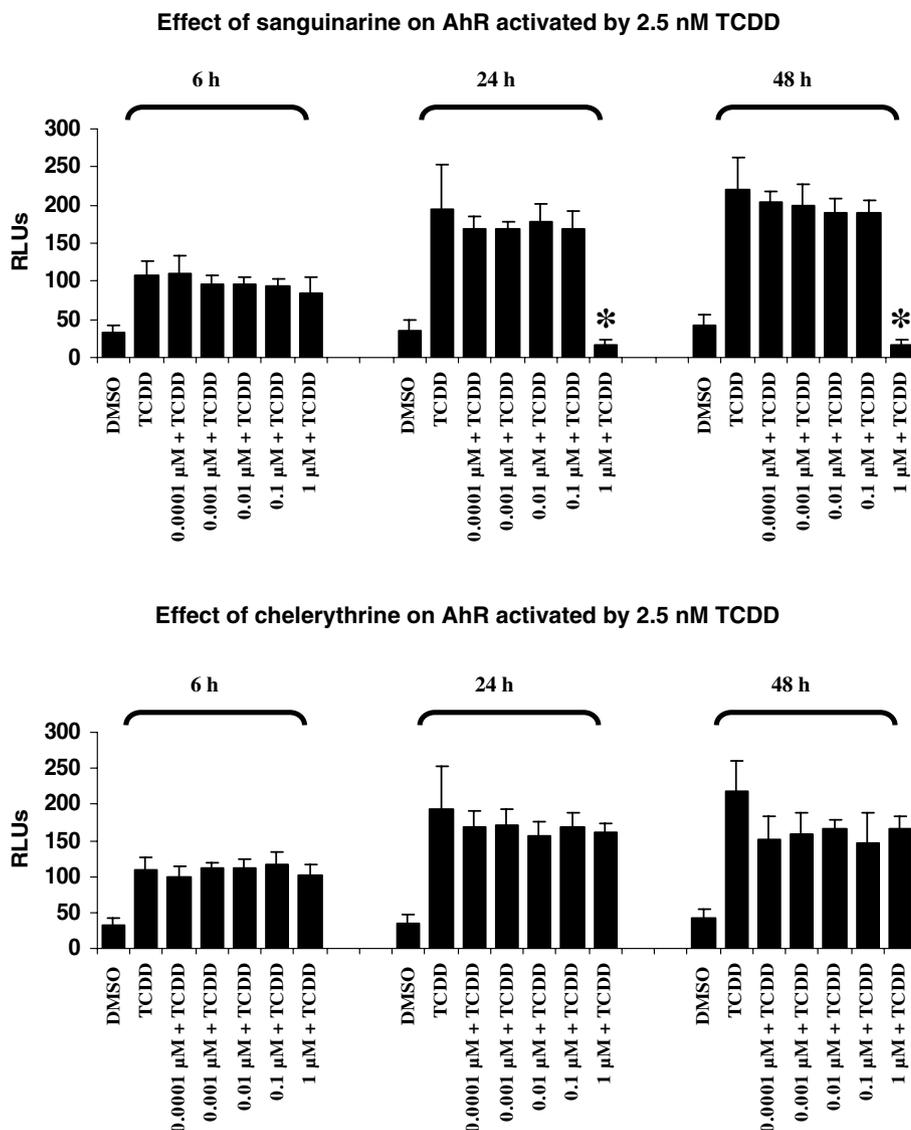


Fig. 6. Effects of sanguinarine and chelerythrine on AhR activated by 2.5 nM TCDD in H4IIE.luc cells. Cells were treated 6, 24 and 48 h with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; final concentration 2.5 nM) in the presence of sanguinarine (SA; final concentrations 0.0001 μM, 0.001 μM, 0.01 μM, 0.1 μM, 1 μM), chelerythrine (CHE; final concentrations 0.0001 μM, 0.001 μM, 0.01 μM, 0.1 μM, 1 μM) and/or DMSO as vehicle for control. Luciferase activity was measured by chemiluminescence as described in Section 2. Bar graphs represent means \pm SD from three independent experiments. Within each experiment, four parallel measurements were acquired. * – the value significantly different from the TCDD value at $p < 0.05$.

tation (Dvorak et al., 2005). Indeed, sanguinarine failed to induce AhR-dependent CYP1A1 expression in human hepatoma cells HepG2 in our experiments (Zdarilova et al., 2006). To resolve whether sanguinarine activates AhR or not, we analyzed the effects of sanguinarine in rat hepatoma cells stably transfected with dioxin-responsive elements fused to luciferase gene (H4IIE.luc cells). This experimental model is routinely used for the screening of AhR activators (Hilscherova et al., 2000). The data presented here clearly show that sanguinarine and/or chelerythrine in submicromolar concentrations do not activate AhR in H4IIE.luc cells. In contrast, we have described elsewhere strong activation of AhR by structurally close alkaloid berberine in HepG2 and H4IIE.luc cells (Vrzal et al., 2005).

Based on the cytotoxicity assays we have challenged cells with submicromolar concentrations of tested QBAs. The rationale for testing QBAs in submicromolar concentration is further supported by recent pharmacokinetic study on rats (Psotova et al., 2006). There is a perpetual disputation among the scientists about the choice of QBAs concentrations. In certain cases it is a philosophic rather than scientific debate. Should we apply the substances to the cells in “non-toxic” concentration, i.e. in concentration which do not alter normal cell functions? What is the rationale for the treatment of the cells with “inactive” concentrations? Is there always strict difference between “non-toxic” and “inactive” concentration? For instance, QBAs have been respected for a long time as inhibitors of protein kinase C (PKC). Treatment of the cells with 4 μM

chelerythrine effectively impaired TCDD mediated activation of AhR, because active PKC is essential for proper AhR activation (Long et al., 1998). On the other hand, QBAs in concentration 2–5 μM cause 50% decrease in viability after 3–6 h in many cell lines (Walterova et al., 1995). Our findings presented here imply that QBAs in sub-micromolar concentration do not affect TCDD-mediated activation of AhR.

In conclusion, we demonstrate that at the doses investigated (i) sanguinarine and chelerythrine are not activators of Ah receptor-mediated transcriptional activation of *Cyp1a1*; (ii) Sanguinarine and chelerythrine do not affect TCDD-mediated AhR activation. Our data favor rather for inactivity or modest inhibitory effect of QBAs on AhR signaling pathways *in vitro* than for the activation of the receptor. Taken together with concentrations of QBAs occurring *in vivo* (Kosina et al., 2004), the use of products containing sanguinarine and/or chelerythrine has low toxicological potential in terms of the interaction with AhR signaling pathways.

Acknowledgements

This research was supported by grant MSM 6198959216 from the Ministry of Education, Youth and Sports of the Czech Republic, and by grant GACR 303/04/P074 from the Grant Agency of the Czech Republic.

References

- Dostal, J., Taborska, E., Slavik, J., 1992. Preparative column chromatography of quaternary benzophenanthridine alkaloids of *Dicranostigma lactucoides*. *Fitoterapia* 63, 67–70.
- Dvorak, Z., Modriansky, M., Simanek, V., Ulrichova, J., Vicar, J., Vrba, J., Walterova, D., 2005. Sanguinarine activates polycyclic aromatic hydrocarbon associated metabolic pathways in human oral keratinocytes and tissues. *Toxicol Lett.* 158, 164–165.
- Giuliana, G., Pizzo, G., Milici, M.E., Musotto, G.C., Giangreco, R., 1997. In vitro antifungal properties of mouthrinses containing antimicrobial agents. *J Periodontol.* 68, 729–733.
- Hilscherova, K., Machala, M., Kannan, K., Blackenship, A.L., Giesy, J.P., 2000. Cell Bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Environ. Sci. Pollut. Res.* 7, 159–171.
- Hong, S.J., Jeong, S.S., Song, K.B., 2005. Effects of sanguinarine in fluoride-containing dentifrices on the remineralisation of subsurface carious lesion *in vitro*. *Int. Dent. J.* 55, 128–132.
- Karp, J.M., Rodrigo, K.A., Pei, P., Pavlick, M.D., Andersen, J.D., McTigue, D.J., Fields, H.W., Mallery, S.R., 2005. Sanguinarine activates polycyclic aromatic hydrocarbon associated metabolic pathways in human oral keratinocytes and tissues. *Toxicol. Lett.* 158, 50–60.
- Kosina, P., Walterova, D., Ulrichova, J., Lichnovsky, V., Stiborova, M., Rydlova, H., Vicar, J., Krecman, V., Brabec, M.J., Simanek, V., 2004. Sanguinarine and chelerythrine: assessment of safety on pigs in ninety days feeding experiment. *Food Chem. Toxicol.* 42, 85–91.
- Long, W.P., Pray-Grant, M., Tsai, J.C., Perdew, G.H., 1998. Protein kinase C activity is required for aryl hydrocarbon receptor pathway-mediated signal transduction. *Mol. Pharmacol.* 53, 691–700.
- Maiti, M., Nandi, R., Chaudhuri, K., 1982. Sanguinarine: a monofunctional intercalating alkaloid. *FEBS Lett.* 142, 280–284.
- Psotova, J., Klejdus, B., Vecera, R., Kosina, P., Kuban, V., Vicar, J., Simanek, V., Ulrichova, J., 2006. A liquid chromatographic-mass spectrometric evidence of dihydrosanguinarine as a first metabolite of sanguinarine transformation in rat. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 830, 165–172.
- Sanderson, J.T., Aarts, J.M., Brouwer, A., Froese, K.L., Denison, M.S., Giesy, J.P., 1996. Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-*O*-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. *Toxicol Appl Pharmacol.* 137, 316–325.
- Schmeller, T., Latz-Bruning, B., Wink, M., 1997. Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. *Phytochemistry* 44, 257–266.
- Southon, I.W., Buckingham, J., 1989. *Dictionary of Alkaloids*. Chapman and Hall, London.
- Stiborova, M., Simanek, V., Frei, E., Hobza, P., Ulrichova, J., 2002. DNA adduct formation from quaternary benzo[*c*]phenanthridine alkaloids sanguinarine and chelerythrine as revealed by the 32 P-postlabeling technique. *Chem. Biol. Interact.* 140, 231–242.
- Stoscheck, C.M., 1990. Quantitation of protein. *Methods Enzymol.* 182, 50–68.
- Vrba, J., Kosina, P., Ulrichova, J., Modriansky, M., 2004. Involvement of cytochrome P450 1A in sanguinarine detoxication. *Toxicol. Lett.* 151, 375–387.
- Vrzal, R., Zdarilova, A., Ulrichova, J., Blaha, L., Giesy, J.P., Dvorak, Z., 2005. Activation of the aryl hydrocarbon receptor by berberine in HepG2 and H4IIE cells: Biphasic effect on CYP1A1. *Biochem. Pharmacol.* 70, 925–936.
- Walterova, D., Ulrichova, J., Valka, I., Vicar, J., Vavreckova, C., Taborska, E., Harjrader, R.J., Meyer, D.L., Cerna, H., Simanek, V., 1995. Benzo[*c*]phenanthridine alkaloids sanguinarine and chelerythrine: biological activities and dental care applications. *Acta Univ. Palacki Olomuc. Fac. Med.* 139, 7–16.
- Wang, B.H., Lu, Z.X., Polya, G.M., 1997. Inhibition of eukaryote protein kinases by isoquinoline and oxazine alkaloids. *Planta Med.* 63, 494–498.
- Williams, M.K., Dalvi, S., Dalvi, R.R., 2000. Influence of 3-methylcholanthrene pretreatment on sanguinarine toxicity in mice. *Vet. Hum. Toxicol.* 42, 196–198.
- Zdarilova, A., Vrzal, R., Rypka, M., Ulrichova, J., Dvorak, Z., 2006. Investigation of sanguinarine and chelerythrine effects on CYP1A1 expression and activity in human hepatoma cells. *Food Chem. Toxicol.* 44, 242–249.