Journal of Theoretical Biology 357 (2014) 268–271

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

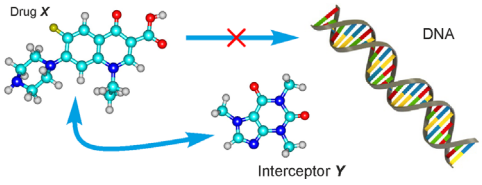
Letter to Editor

Quantitative correlation of the *in vitro* biological effect with parameters of molecular complexation in mutagen-interceptor systems

HIGHLIGHTS

- The quantitative link between biological and physico-chemical parameters is found.
- correction to the known correlation of biological and phys-chem data is suggested.
- Mutagen-interceptor systems well match the theory of interceptor–protector action.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
IQ-type mutagens
Mutagenic test
Interceptor mechanism
Hetero-association

ABSTRACT

According to the theory of interceptor–protector action a quantitative link between the physico-chemical parameters of molecular complexation and *in vitro* biological effect in aromatic drug-interceptor systems must exist. In the present communication such link between relative change in mutagenicity of IQ-type aromatic mutagens on addition of aromatic interceptor molecules with equilibrium hetero-association constants of mutagen-interceptor complexation has been found using the published *in vitro* data in bacteria cell systems.

1. Introduction

Biological interaction, observed when different aromatic biologically active compounds are used in combination, has been known for a long time. The most well-studied combinations of aromatic molecules are DNA-binding aromatic drugs with xanthines, riboflavin and chlorophyllin (see for reviews Evstigneev (2010, 2013); Woziwodzka et al. (2013)). It is considered that the administration of certain aromatic drug (e.g. antitumor antibiotic) along with other aromatic compound, called 'interceptor' (e.g. caffeine or chlorophyllin), can be used for in situ regulation of the toxicity induced by antibiotic during chemotherapy, or, in some cases, result in amplification of medico-biological effects of the antibiotic (Traganos et al., 1991; Piosik et al., 2002).

Numerous investigations carried out mainly on the *in vitro* level have reported that the mechanism of the biological interaction in drug-interceptor systems may be interpreted in terms of non-covalent complexation between the molecular components present in the mixture, *viz.* the drug-interceptor hetero-association, and drug-DNA and interceptor-DNA complexation (Evstigneev, 2010, 2013; Woziwodzka et al., 2013; Traganos et al., 1991; Piosik et al., 2002). These interactions lie behind the two fundamental molecular mechanisms of biological interaction induced when DNA-binding aromatic drugs are used in combination, *viz.* the interceptor (i.e. the drug-interceptor hetero-association) and protector (i.e. competition of drug and interceptor for DNA-binding sites) mechanisms. The generalization of this view has been accomplished within the framework of the theory of interceptor–protector action (the IPA theory) (Evstigneev, 2010; Evstigneev et al., 2008; Buchelnikov et al., 2012), which aims to find a quantitative link between the *in vitro* biological data and parameters of physico-chemical interactions (concentrations and equilibrium complexation constants).

So far the IPA theory has been successfully applied to quantification of relative change in apoptosis in human leukemia cell lines induced by administration of antitumor antibiotics together with caffeine (Evstigneev et al., 2006, 2008, 2011). However, to date one more set of biological data, well matching the basic postulates of the IPA theory, has been accumulated, *viz.* the antimutagenic action of the interceptor molecules towards aromatic mutagens in bacteria cell systems (see for review Piosik et al. (2003); Woziwodzka et al. (2011); Dashwood and Guo (1993)).

http://dx.doi.org/10.1016/j.jtbi.2014.06.016
0022-5193/

In the present letter we aim to testify the existence of quantitative link between the biological and physico-chemical parameters in mutagen-interceptor systems based on the IPA theory.

2. Discussion

2.1. General approach to quantitation of the in vitro biological data

Let X be the main drug exerting its biological effect via complexation with DNA (N), and Y be the interceptor molecule which does not exert any biological effect (or its biological effect does not interfere with X) and be able to form hetero-complexes with X. Let φ be the measureable response of a biological system to the presence of both X and Y, whereas φ_0 and φ_c be the responses of this system (e.g. percentage of apoptotic cells or mutagenicity) to the presence of X only, and in the absence of both drugs, respectively (i.e. positive and negative control values). The key quantity in the IPA theory is the A_D factor

$$A_D = \frac{\varphi_0 - \varphi}{\varphi_0 - \varphi_c}, \tag{1}$$

which stands for relative change in biological effect of X on addition of Y (examples of linking the A_D factor to biological data are given in Evstigneev et al. (2006, 2008, 2011)).

The principal assumption associated with the A_D factor is a proportionality between the mole fraction of X-DNA complexes, f_C^X, and the observed biological effect, φ. As a consequence of this assumption, the relative change of biological effect of X on addition of Y (i.e. the A_D factor) may be quantitatively expressed in terms of relative change in mole fractions of X-DNA complexes as (Evstigneev et al., 2006, 2008)

$$A_D = \frac{f_{C(0)}^X - f_C^X}{f_{C(0)}^X}, \tag{2}$$

where $f_{C(0)}^X$ is the mole fraction of X-DNA complexes in the absence of interceptor.

Such definition of the A_D factor sets up a direct link between the biological (A_D or φ) and physico-chemical (f_C^X) parameters. In order to utilize this link, the method of computation of the mole fractions is needed.

The mole fractions in Eq. (2) may be found from the solution of mass balance equations for the X-Y-DNA system, given in Evstigneev et al. (2008) in the most simple form as

$$\begin{cases} x_1 + K_h x_1 y_1 + K_{XN} x_1 N_1 = x_0 \\ y_1 + K_h x_1 y_1 + K_{YN} y_1 N_1 = y_0 \\ N_1 + K_{XN} x_1 N_1 + K_{YN} y_1 N_1 = N_0 \end{cases}, \tag{3}$$

where x_1, y_1, N_1 are the concentrations of free (non-complexed) drug and interceptor molecules, and DNA binding sites, respectively; x_0, y_0, N_0 are their total concentrations commonly associated with the so-called quasi-physiological conditions ($N_0 = x_0$, $y_0 \gg x_0$ Evstigneev et al. (2006, 2008, 2011)); K_h, K_{XN}, K_{YN} are the equilibrium constants of hetero-association, X-DNA and Y-DNA binding, respectively.

Solution of Eq. (3) with respect to x_1, y_1, N_1 enables one to compute the mole fractions in Eq. (2) as

$$f_C^X = \frac{K_{XN} x_1 N_1}{x_0}\bigg|_{\substack{K_{YN} \neq 0 \\ K_h \neq 0}}, \; f_{C(0)}^X = \frac{K_{XN} x_1 N_1}{x_0}\bigg|_{\substack{K_{YN} = 0 \\ K_h = 0}}. \tag{4}$$

So, the set of Eqs. (2)–(4) in the IPA theory provides a quantitative link between the biological and physico-chemical data, which will be explored below against the mutagenic test in bacteria cell systems.

2.2. Evaluation of biological data from mutagenic test in mutagen-chlorophyllin system

Chlorophyllin (CHL) has long been recognized as a molecule exerting pronounced antimutagenic potency against various aromatic DNA-acting mutagens (Dashwood and Guo 1993; Pietrzak et al., 2008). This effect has been interpreted in terms of formation of non-covalent hetero-complexes between the mutagen and CHL resulting in lower accessibility of the mutagen to DNA (Woziwodzka et al., 2013; Dashwood and Guo 1993; Pietrzak et al., 2008) (which is the interceptor mechanism falling within the competency of the IPA theory).

The most representative example of the mutagen-chlorophyllin systems matching the interceptor hypothesis is the antimutagenic action of CHL against imidazo-quinoline type amines (IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline) in *Salmonella typhimurium* strain TA98 reviewed in Dashwood and Guo (1993) by Dashwood. Statistical treatment of large IQ-CHL dataset had led to a conclusion about a linear correlation between the antimutagenic potency of chlorophyllin, I_{50} (which is the chlorophyllin concentration needed for 50% suppression of mutagenicity in the cell culture treated with IQ mutagens), and the hetero-association constant of the formation of IQ-CHL complexes, K_h

$$I_{50} \propto K_h. \tag{5}$$

Let us find out whether the Eq. (5) may be derived as a partial case of the IPA theory.

It is known that the IQ-type mutagens directly act on DNA by means of formation of covalent adducts (Turesky et al., 1992) which suggests the absence of competition between IQ (X) and chlorophylline (Y) and, hence, no contribution from the protector mechanism (i.e. $K_{YN}=0$). Corresponding simplification of Eq. (3) yields

$$\begin{cases} x_1 + K_h x_1 y_1 + \frac{K_{XN} x_1}{1 + K_{XN} x_1} N_0 = x_0 \\ y_1 + K_h x_1 y_1 = y_0 \end{cases}. \tag{6}$$

The solution of Eq. (6) against x_1 leads to a third-order algebraic equation

$$K_h K_{XN} x_1^3 + (K_h + K_{XN} + K_h K_{XN}(y_0 + N_0 - x_0)) x_1^2 + (1 + K_h(y_0 - x_0) + K_{XN}(N_0 - x_0)) x_1 - x_0 = 0. \tag{7}$$

The typical non-toxic concentrations of the IQ-type mutagens in the mutagen test fall in the nM-μM range (Woziwodzka et al., 2011; Dashwood and Guo 1993), so the second- and third-order terms in Eq. (7) may be neglected. It allows one to get expression for x_1 to be further substituted into Eq. (4) for derivation of the f_C^X quantities, required for computation of the A_D factor in Eq. (2). Employing the quasi-physiological conditions ($N_0 = x_0$, $y_0 \gg x_0$ Evstigneev et al. (2006, 2008, 2011)) and performing the set of corresponding mathematical manipulations, the approximate expression for the A_D factor takes the form

$$A_D \approx \frac{K_h y_0}{1 + K_h y_0}. \tag{8}$$

Eq. (8) can be further simplified. Taking into account the typically micromolar chlorophyllin concentrations in the mutagen test, $y_0 \sim$ μM, and the IQ-CHL hetero-association constants, K_h, having $10^3\ \mathrm{M}^{-1}$ order of magnitude (Dashwood and Guo 1993), one can get $K_h y_0 \ll 1$ in Eq. (8) resulting in expression

$$A_D \approx K_h y_0. \tag{9}$$

Recalling that the biological data in Dashwood and Guo (1993) were expressed in the form of the I_{50} factor which is the concentration of chlorophyllin needed for 50% suppression of mutagenicity in the mutagen test, the following substitutions are valid, *viz.* $y_0 \equiv I_{50}$ and $A_D \equiv 1/2$. Further transformation of Eq. (9) results in the approximate

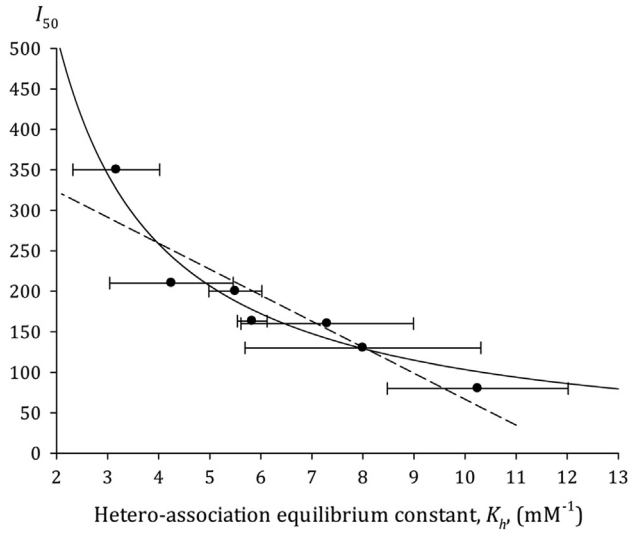


Fig. 1. Correlation of the I_{50} factor (chlorophyllin concentration resulting in 50% inhibition of mutagenicity of IQ-type mutagens) with the IQ-CHL hetero-association constants: dashed line is linear correlation, $R^2=0.82$; solid line is a hyperbolic correlation according to Eq. (10), $R^2=0.94$. The data for I_{50} and K_h were taken from Fig. 4 in (Dashwood and Guo, 1993).

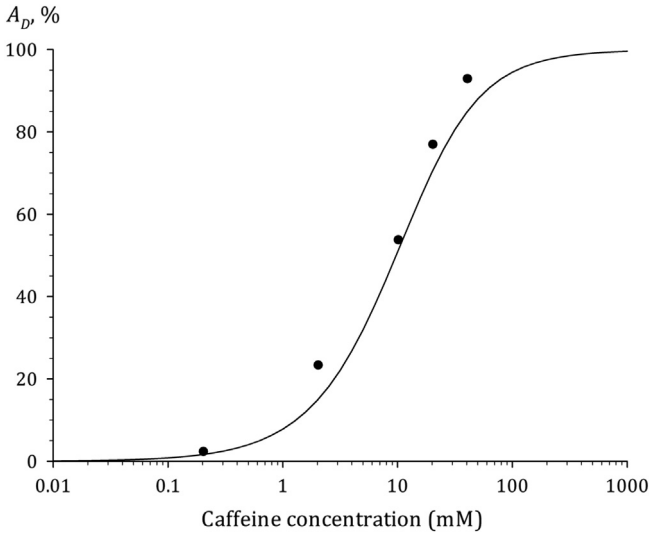


Fig. 2. Dependence of the A_D factor in IQ-CAF system on caffeine concentration: points – A_D recalculated from biological data given in (Woziwodzka et al., 2011) according to Eq. (1); solid line – A_D computed from Eqs. (2) and (3) under the sole action of the interceptor mechanism.

expression for the link between the I_{50} and K_h factors:

$$I_{50}=\frac{B}{K_h}, \tag{10}$$

where B is certain constant.

Fitting of the Dashwood data (Fig. 4a in Dashwood and Guo (1993)) with Eq. (10) shown in Fig. 1 gives much better goodness of fit ($R^2=0.94$) as compared with the linear correlation [see Eq. (5)] ($R^2=0.82$) initially reported in the cited paper. It means that the hyperbolic dependence of biological effect (expressed in the form of the I_{50} factor) and the physico-chemical parameter of interaction (expressed in the form of K_h) reflects the fundamental action of the interceptor mechanism in its 'pure' form when the other possible mechanisms (such as the protector, or any other) contribute negligibly to experimental observable φ. This result is also supported by the fact that the equilibrium complexation constants have much higher impact on the value of the A_D factor as compared with other parameters appearing in the system of mass balance equations (such as Eq. (3)) (Buchelnikov et al., 2013).

2.3. Evaluation of biological data from mutagenic test in mutagen-caffeine system

The success in description of the link between the biological and physico-chemical data in mutagen-chlorophyllin systems within the framework of the IPA theory suggests that similar correlation should be observed for other interceptors as well. With the use of mutagen test in *Vibrio harveyi* strain A16 the authors of Woziwodzka et al. (2011) measured the dependence of mutagenic activity of the IQ-type amines on the concentration of various xanthines, y_0, among which the caffeine (CAF) had exerted the most pronounced effect (Fig. 8a in Woziwodzka et al. (2011)). Fig. 2 contains the biological data recalculated into A_D units according to Eq. (1), and the $A_D(y_0)$ dependence computed from Eqs. (2) and (3) under the sole action of the interceptor mechanism (i.e. $K_{YN}=0$) (the magnitudes of the complexation parameters were taken from Woziwodzka et al. (2011) and the estimated magnitude of the IQ-DNA binding was taken as $K_{XN}=2970\ M^{-1}$ from Sartorius and Schneider (1997)). Small variation of the binding parameters within 10% range enabled to achieve very good fitting of experimental data (solid line in Fig. 2) suggesting that the IQ-CAF systems follow the interceptor mechanism in the same way as the IQ-CHL systems discussed above.

3. Conclusions

Based on the results of investigation it may be concluded that if the mechanism of change of biological effect under the use of aromatic drugs in combination follows the interceptor hypothesis, this situation may be described within the framework of the interceptor–protector theory which quantifies the link between the *in vitro* biological data and the physico-chemical parameters of molecular complexation. Previously the existence of such a link was confirmed for antibiotic-interceptor combinations using *in vitro* data in leukemia cell lines (see the introductory section). In the present work we report the existence of such link with respect to mutagen-interceptor combinations in bacteria cell systems. These results are important in terms of managing drug's response by changing the physico-chemical parameters of molecular complexation and extend scientific background of rational drug design.

References

Buchelnikov, A.S., Hernandez Santiago, A.A., Gonzalez Flores, M., Vazquez Ramirez, R., Davies, D.B., Evstigneev, M.P., 2012. General analysis of competitive binding in drug–interceptor–DNA systems. Eur. Biophys. J. 41, 273–283, http://dx.doi.org/10.1007/s00249-011-0783-3.

Buchelnikov, A.S., Evstigneev, V.P., Rodríguez Oropeza, L.E., Evstigneev, M.P., 2013. On the reliability of quantitation of biological effect in drug-interceptor-DNA systems. Eur. Biophys. J. 42, 315–319, http://dx.doi.org/10.1007/s00249-013-0893-1.

Dashwood, R., Guo, D., 1993. Antimutagenic potency of chlorophyllin in the Salmonella assay and its correlation with binding constants of mutagen-inhibitor complexes. Environ. Mol. Mutagen. 22, 164–171, http://dx.doi.org/10.1002/em.2850220309.

Evstigneev, M.P., 2010. Dna-Binding Aromatic Drug Molecules: Physico-Chemical Interactions and their Biological Roles. Lambert Academic Publishing, Saarbrücken.

Evstigneev, M.P., 2013. Physicochemical mechanisms of synergistic biological action of combinations of aromatic heterocyclic compounds. Org. Chem. Int. 2013, http://dx.doi.org/10.1155/2013/278143 (Article ID 278143).

Evstigneev, M.P., Khomich, V.V., Davies, D.B., 2006. Complexation of anthracycline drugs with DNA in the presence of caffeine. Eur. Biophys. J. 36, 1–11, http://dx.doi.org/10.1007/s00249-006-0071-9.
Evstigneev, M.P., Lantushenko, A.O., Evstigneev, V.P., Mykhina, Yu.V., Davies, D.B., 2008. Quantitation of the molecular mechanisms of biological synergism in a mixture of DNA-acting aromatic drugs. Biophys. Chem. 132, 148–158, http://dx.doi.org/10.1016/j.bpc.2007.11.001.
Evstigneev, M.P., Mosunov, A.A., Evstigneev, V.P., Parkes, H.G., Davies, D.B., 2011. Quantification of the interceptor action of caffeine on the in vitro biological effect of the anti-tumour agent topotecan. Eur. Biophys. J. 40, 969–980, http://dx.doi.org/10.1007/s00249-011-0712-5.
Pietrzak, M., Halicka, H.D., Wieczorek, Z., Wieczorek, J., Darzynkiewicz, Z., 2008. Attenuation of acridine mutagen ICR-191—DNA interactions and DNA damage by the mutagen interceptor chlorophyllin. Biophys. Chem. 135, 69–75, http://dx.doi.org/10.1016/j.bpc.2008.03.004.
Piosik, J., Zdunek, M., Kapuściński, J., 2002. The modulation by xanthines of the DNA-damaging effect of polycyclic aromatic agents: Part II. The stacking complexes of caffeine with doxorubicin and mitoxantrone. Biochem. Pharmacol. 63, 635–646, http://dx.doi.org/10.1016/s0006-2952(01)00903-0.
Piosik, J., Ulanowska, K., Gwizdek-Wiśniewska, A., Czyż, A., Kapuściński, J., Węgrzyn, G., 2003. Alleviation of mutagenic effects of polycyclic aromatic agents (quinacrine mustard, ICR-191 and ICR-170) by caffeine and pentoxifylline. Mutat. Res., Fundam. Mol. Mech. Mutagen. 530, 47–57, http://dx.doi.org/10.1016/s0027-5107(03)00136-2.
Sartorius, J., Schneider, H.-J., 1997. Intercalation mechanisms with ds-DNA: binding modes and energy contributions with benzene, naphthalene, quinoline and indole derivatives including some antimalarials. J. Chem. Soc., Perkin Trans. 2 (11), 2319–2328, http://dx.doi.org/10.1039/A702628E.
Traganos, F., Kapuściński, J., Darzynkiewicz, Z., 1991. Caffeine modulates the effects of DNA-intercalating drugs in vitro: a flow cytometric and spectrophotometric analysis of caffeine interaction with novantrone, doxorubicin, ellipticine, and the doxorubicin analogue AD198. Cancer Res. 51, 3682–3689.
Turesky, R.J., Rossi, S.C., Welti, D.H., Lay, J.O., Kadlubar, F.F., 1992. Characterization of DNA adducts formed in vitro by reaction of *N*-hydroxy-2-amino-3-methylimidazo(4,5-f) quinoline and *N*-hydroxy-2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline at the C-8 and N^2 atoms of guanine. Chem. Res. Toxicol. 5, 479–490, http://dx.doi.org/10.1021/tx00028a005.
Woziwodzka, A., Gwizdek-Wiśniewska, A., Piosik, J., 2011. Caffeine, pentoxifylline and theophylline form stacking complexes with IQ-type heterocyclic aromatic amines. Bioorg. Chem. 39, 10–17, http://dx.doi.org/10.1016/j.bioorg.2010.11.001.
Woziwodzka, A., Gołuński, G., Piosik, J., 2013. Heterocyclic aromatic amines heterocomplexation with biologically active aromatic compounds and its possible role in chemoprevention. ISRN Biophys. 2013, http://dx.doi.org/10.1155/2013/740821 (Article ID 740821).

Anatoly S. Buchelnikov
Department of Biology and Chemistry, Belgorod State University, Belgorod 308015, Russia

Maxim P. Evstigneev*
Department of Biology and Chemistry, Belgorod State University, Belgorod 308015, Russia
Department of Physics, Sevastopol National Technical University, Sevastopol 299053, Russia
E-mail address: max_evstigneev@mail.ru

Available online 24 June 2014

* Corresponding author at: Department of Physics, Sevastopol National Technical University, Sevastopol 299053, Russia. Tel./fax: +7 9205641945.