ESTROGEN RECEPTOR BINDING AFFINITY OF FOOD CONTACT MATERIAL COMPONENTS ESTIMATED BY QSAR

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SUMMARY

Aim: The presented work characterized components of food contact materials (FCM) with potential to bind to estrogen receptor (ER) and cause adverse effects in the human organism.

Methods: The QSAR Toolbox, software application designed to identify and fill toxicological data gaps for chemical hazard assessment, was used. Estrogen receptors are much less of a lock-and-key interaction than highly specific ones. The ER is nonspecific enough to permit binding with a diverse array of chemical structures. There are three primary ER binding subpockets, each with different requirements for hydrogen bonding.

Results: More than 900 compounds approved as of FCM components were evaluated for their potential to bind on ER. All evaluated chemicals were subcategorized to five groups with respect to the binding potential to ER: very strong, strong, moderate, weak binder, and no binder to ER. In total 46 compounds were characterized as potential disturbers of estrogen receptor.

Conclusion: Among the group of selected chemicals, compounds with high and even very high affinity to the ER binding subpockets were found. These compounds may act as gene activators and cause adverse effects in the organism, particularly during pregnancy and breast-feeding. It should be considered to carry out further in vitro or in vivo tests to confirm their potential to disturb the regulation of physiological processes in humans by abnormal ER signaling and subsequently remove these chemicals from the list of approved food contact materials.

Key words: QSAR, estrogen receptor, chemicals, food contact materials

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INTRODUCTION

In 1958 Elwood Jensen demonstrated the existence of an estrogen receptor (ER) (1), the corresponding gene was cloned in 1985. In 1996 estrogen receptor beta (ERβ) was cloned from the rat prostate and ovary (2). Reproductive tissues (uterus, ovary), breast, kidney, bone, white adipose tissue, and liver are the places where estrogen receptor alfa (ERα) is mainly expressed, while expression of ERβ is found in the ovary, central nervous system (CNS), cardiovascular system, lung, male reproductive organs, prostate, colon, kidney, and the immune system. ERs are found mainly in the nucleus, but also in the cytoplasm and mitochondria.

The ERα and ERβ genes are located on different chromosomes, 6q25.1 and 14q23.2, respectively. ERs are composed of three functional domains: the NH2-terminal domain (NTD), the DNA-binding domain (DBD), and the COOH-terminal ligand-binding domain (LBD). The NTD encompasses a ligand independent activation function (AF1) domain involved in transcriptional activation of target genes, and with only 16% similarity between ERα and ERβ. The DBD is highly conserved between ERα and ERβ with 97% amino acid identity and mediates sequence-specific binding of ERs to DNA sequences in target genes denoted estrogen-responsive elements (EREs). In contrast, the LBDs of ERα and ERβ show a 59% overall amino acid sequence identity yet the ligand-binding pockets of the two subtypes show only minor differences in structure (3).

Estrogens have been distinctly shown to regulate glucose and lipid metabolism using either models of estrogen-/ER-depletion or estrogen application/replacement. Estrogen deficiency promotes metabolic dysfunction predisposing to type 2 diabetes (T2D), obesity, and the metabolic syndrome. In rodents it has been demonstrated that aromatase is the key enzyme of estrogen production. Knockout (ArKO) mice display insulin resistance (IR), impaired glucose tolerance (IGT), and increased abdominal fat, which are reversible by 17β-estradiol (E2) treatment (4).

The ER is nonspecific enough to permit binding with a diverse array of chemical structures. There are four primary ER binding subpockets (5, 6), each with different requirements for hydrogen bonding (Fig. 1–3). Steroidal compounds usually interact at two points within the ER using two hydrogen-bonding groups. However, there are also chemicals with one hydrogen-bonding group that bind to the ER and cause subsequent gene activation.

The aim of the work was to characterize chemicals with potential to bind to ER and cause adverse effects in the organism. Over nine hundred compounds used in food contact materials (EU Regulation No 10/2011 on plastic materials and articles) (7) were analyzed using QSAR and computational chemistry.

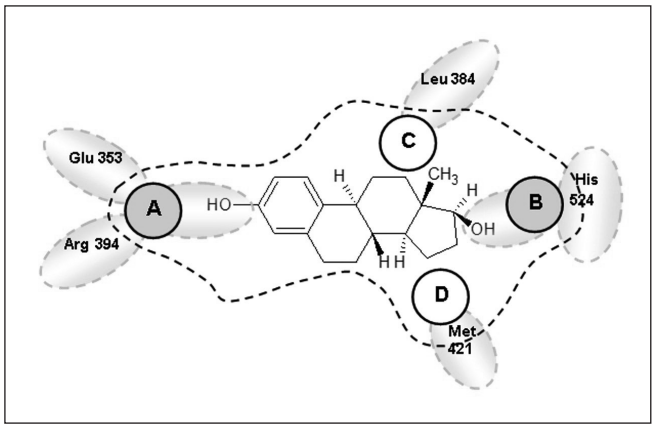

Fig. 1. *A-B interaction of compound with ER binding site (17β-Estradiol).*

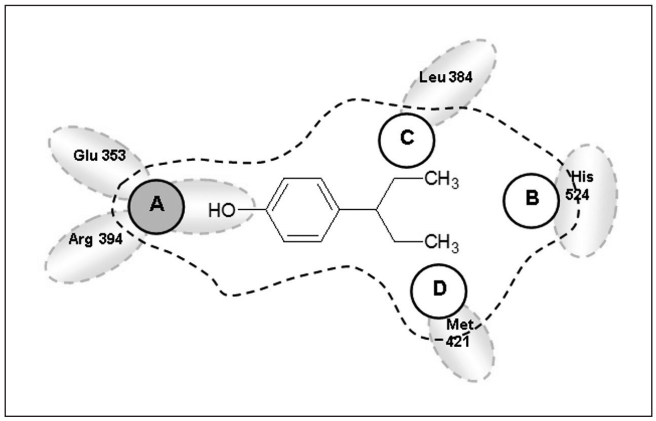

Fig. 2. *A-site only interaction of compound with ER binding site.*

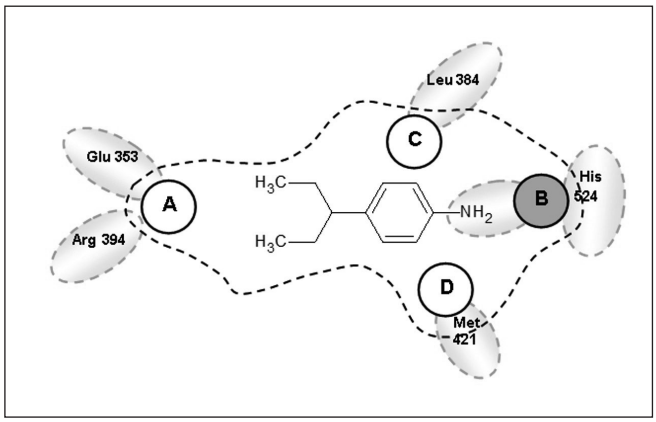

Fig. 3. *B-site only interaction of compound with ER binding site.*

MATERIALS AND METHODS

The QSAR Toolbox software application designed to identify and fill toxicological data gaps for chemical hazard assessment, was used *.

Compounds were subcategorized to groups using cascade of queries. The first query in the tree is whether the chemical contains a cycle. If not, the chemical is considered within the domain of the ER binding model, but at the same time the chemical is predicted to be inactive in the ER-mediated pathway. The second query is aimed to determine if the chemical belongs to one of the subgroups in which all tested members so far did not competitively bind to ER. For the majority of chemicals, the presence of a charge in the molecule, absence of hydrogen bonding group, or inappropriate geometry explains the failure of these chemicals to bind to ER. Chemicals which meet third query requirements are these with greatest binding affinity to the ER receptors. The primary feature for high ER binding activity is to have two oxygen atoms available for hydrogen bonding at the specific distances dictated by the ER receptor. Molecular weight and partition coefficient of evaluated compounds are also considered.

More than 900 compounds from the list of FCM were evaluated on their potential to bind on ER using QSAR Toolbox.

RESULTS

All evaluated chemicals were subcategorized to five groups with respect to the binding potential to ER: very strong, strong, moderate, weak binder, and no binder to ER. In total 46 compounds were characterized as potential disturbers of estrogen receptor (Table 1).

The group of very strong binders to ER includes 7 chemicals. These compounds can significantly disturb the ER and can cause number of adverse effects in the organism. Their presence in the list of food contact materials should be questioned, evaluated and proper decision should be adopted. These substances represent a real threat to the health of the population, especially for children and nursing mothers.

The group of strong binders to ER includes 13 chemicals. These compounds can also significantly disturb the ER and may cause a number of adverse effects in the organism. Their presence in the list of food contact materials should be also questioned and re-evaluated as these substances may also represent a potential threat to the health of the sensitive population.

The group of moderate binders to ER includes 7 chemicals. These compounds have a limited potential to disturb the ER and may also cause adverse effects in the organism. Although the total effect is not so strong, continuous disturbing of the ER can lead to chronic problems with human health where the reason is not known.

The group of weak binders to the ER includes 19 chemicals. These compounds have a weak potential to disturb the ER. Their effect on human health is rather questionable, only in case of very high exposition they can significantly disturb the ER.

DISCUSSION

Estrogen receptors alpha and beta are transcription factors that are involved in the regulation of many complex physiological processes in humans. Abnormal ER signaling leads to development of a variety of diseases, such as cancer, metabolic and cardiovascular disease, neurodegeneration, inflammation (8), and osteoporosis (3). Chemical binding to the ER is one of the significant mechanisms interfering with process of reproduction (9, 10).

*http://www.qsartoolbox.org/home

Table 1. *The list of evaluated chemicals showing affinity to the ER binding site*

No	CAS No	Substance name	ER binding affinity
1	59-02-9	α-tocopherol	WA
2	69-72-7	salicylic acid	WA
3	80-05-7	2,2-bis(4-hydroxyphe-nyl)propane (Bisphenol A)	VSA
4	80-09-1	4,4'-dihydroxydiphenyl sulphone (Bisfenol S)	MA
4	84-74-2	phthalic acid, dibutyl ester	MA
6	85-68-7	phthalic acid, benzyl butyl ester	MA
7	87-18-3	salicylic acid, 4-tert-butylphenyl ester	SA
8	88-68-6	2-aminobenzamide	WA
9	92-88-6	4,4'-dihydroxybiphenyl	MA
10	94-13-3	4-hydroxybenzoic acid, propyl ester	MA
11	95-48-7	o-cresol	WA
12	96-69-5	4,4'-thiobis(6-tert-butyl-3-methylphenol)	VSA
13	97-23-4	2,2'-dihydroxy–5,5'-dichlorodiphenyl-methane	VSA
14	97-53-0	eugenol	WA
15	98-54-4	4-tert-butylphenol	WA
16	99-76-3	4-hydroxybenzoic acid, methyl ester	WA
17	99-96-7	p-hydroxybenzoic acid	WA
18	103-90-2	N-(4-hydroxyphenyl) acetamide	WA
19	106-44-5	p-cresol	WA
20	108-39-4	m-cresol	WA
21	108-45-2	1,3-phenylenediamine	WA
22	108-46-3	1,3-dihydroxybenzene	WA
23	108-91-8	cyclohexylamine	WA
24	108-95-2	phenol	WA
25	119-36-8	salicylic acid, methyl ester	WA
26	120-47-8	4-hydroxybenzoic acid, ethyl ester	WA
27	120-80-9	1,2-dihydroxybenzene	WA
28	121-79-9	gallic acid, propyl ester	SA
29	123-31-9	1,4-dihydroxybenzene	WA
30	131-53-3	2,2'-dihydroxy-4-methoxybenzophenone	VSA
31	131-56-6	2,4-dihydroxybenzophenone	SA
32	131-57-7	2-hydroxy-4-methoxybenzophenone	SA
33	599-64-4	4-cumylphenol	SA
34	611-99-4	4,4'-dihydroxybenzophenone	VSA
35	1034-01-1	gallic acid, octyl ester	SA
36	1166-52-5	gallic acid, dodecyl ester	SA
37	1761-71-3	bis(4-aminocyclohexyl)methane	SA
38	1843-05-6	2-hydroxy-4-n-octyloxybenzophenone	SA
39	2440-22-4	2-(2'-hydroxy-5'-methylphenyl)benzotriazole	SA
40	2855-13-2	1-amino-3-aminomethyl-3,5,5-trimethyl-cyclohexane	MA
41	3293-97-8	2-hydroxy-4-n-hexyloxybenzophenone	SA
42	6864-37-5	3,3'-dimethyl-4,4'-diaminodicyclohexylmethane	SA
43	25013-16-5	tert-butyl-4-hydroxyanisole	MA
44	27955-94-8	1,1,1-tris(4-hydroxyphenol)ethane	VSA
45	47465-97-4	3,3-bis(3-methyl-4-hydroxyphenyl)2-indolinone	VSA
46	147315-50-2	2-(4,6-diphenyl-1,3,5-triazin-2-yl)-5-(hexyloxy)phenol	SA

WA – weak affinity, MA – medium affinity, SA – strong affinity, VSA – very strong affinity

Because of the complexity of the effect of potential ER disturbers, the corresponding in vivo method are time consuming and very expensive, especially in the case of reproductive toxicity studies (Extended One-Generation Reproductive Toxicity Study OECD 443) (11). Fast screening methods or in silico methods are the proper way for testing of hundreds of chemicals to find the group of compounds with the highest potential to cause adverse effect in humans. The QSAR Toolbox is one of the most promising computational software in the field of fast, precious and transparent assessment of variety outcomes. It employs the databases of experimental results, trend analysis and proper categorization of compound to the corresponding group and creates well defined QSAR models with applicability domain.

Compounds used in food contact materials represent one of the most important sources of potential contaminants of foodstuffs. They can freely migrate from the packing material to the meal and then can expose humans. Especially in the case of ER disruptors, the effective concentration of acting compounds could be very low, making these compounds even more dangerous.

CONCLUSION

Based on the measured data and proper categorization the compounds can be placed into groups of inactive chemicals, into "drug-like" groups of chemicals which have high potential for significant ER binding affinity, or into groups of chemicals which may have weak to moderate binding affinity depending on specific properties or structural features.

Over nine hundred compounds used in food contact materials were analyzed using QSAR and computational chemistry. Among the group of selected chemicals, compounds with high and even very high affinity to the ER binding subpockets were found. These compounds may act as gene activators and cause adverse effects in the organism, particularly during pregnancy and breast-feeding. It should be considered to carry out further in vitro or in vivo tests to confirm their potential to disturb the regulation of physiological processes in humans by abnormal ER signaling and subsequently remove these chemicals from the list of food contact materials.

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Conflict of Interests

None declared

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