DNA and protein binding of hepatotoxic metabolites of some parabens

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Abstract: *Parabens are alkyl esters of phydroxybenzoic acid and typically include methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben, isopropylparaben and benzylparaben. Parabens are widely used as preservatives in many foods, cosmetics, toiletries, and pharmaceuticals due to their relatively low toxicity profile and to a long history of safe use. Parabens are generally considered as safe preservatives, since they are rapidly absorbed and metabolized into p-hydroxybenzoic acid, which is less toxic than the parent compounds, and is therefore consumed in large quantities in a daily basis. Because of their widespread use, the potential toxicity of parabens has been studied both in vivo and in vitro to assess a variety of toxicological aspects. The aim of this work was to predict the DNA and protein binding of hepatotoxic metabolites for some parabens by a specialized software.*

Key words: *parabens, hepatotoxicity, metabolites, DNA and protein binding*

INTRODUCTION

Parabens are widely used as preservatives to inhibit microbial growth and extend shelf life of products in food, pharmaceuticals, cosmetics, sunscreens, skin-care products, conditioners,shampoos, soaps and deodorants. By far the most prevalent use of parabens has been in cosmetics. In fact, in 1984 it was estimated that parabens were used in 13,200 formulations [8] but a more recent survey of 215 cosmetic products found that parabens were used in 99% of leave-on products and 77% of rinse-off cosmetics. The total paraben content in paraben-positive cosmetics was found to be 0.01–0.87% [25]. Methyl- and propylparaben are the most commonly used preservatives in cosmetics [14] and the most frequently used preservative system is a combination of methyl and propylparaben [9]. Parabens are allowed in concentrations of up to 1% in cosmetics. The European Community Directive allows the use of parabens with a maximum concentration for each one of 0.4% (w/w) and total maximum concentration 0.8% (w/w) [4, 24].

Metabolism of parabens was studied by treating rats with 100mg of methyl- or propylparaben orally. After oral administration in rats, parabens are absorbed from the gastrointestinal tract and quickly hydrolyzed, to different metabolites, by esterases [7].

Parabens can also be rapidly absorbed by the intact skin [1] and hydrolyzed to *p*-hydroxybenzoic acid and their respective side chains [11]; however studies addressing percutaneous absorption of parabens performed in animals and *in vitro* studies have shown that butylparaben exhibits low penetration, retention in the epidermis and/or hydrolysis in the skin [28].

Although there is increasing concern regarding the effects of parabens, namely, as mentioned previously, a possible role on the increased incidence of breast cancer [5], little data exists on their effects regarding human sperm, although a number of studies with animal models were performed. Rodent exposure to butylparaben [16,17] and propylparaben [18] adversely affected testosterone synthesis and male reproductive function. On the other hand, a recent study performed by the same author exhibited contrary results for methyl- and ethylparaben [19]. Although parabens have weak estrogenic activity, confirmed by positive uterotrophic assays [2,6,10,20,26], these findings are in agreement with studies that indicate that methyl and ethyl esters have less potent *in vitro* and *in vivo* estrogenic activity than either propylparaben or the most potent form, butylparaben [2,26]. In fact, another study performed in fish demonstrated that ethylparaben is approximately sixty times weaker than propyl- and butylparaben [22].

The aim of this work was to predict the DNA and protein binding of hepatotoxic metabolites for some parabens by a specialized software.

MATERIALS AND METHODS

Compounds. Some parabens [3] were investigated which are presented in Table 1.

OECD (Q)SAR Application Toolbox. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox [15].

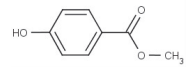
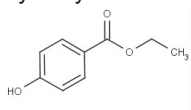
Metabolic pathways documented for 200 organic chemicals in different mammals are stored in a database format that allows easy computer-aided access to the metabolism information. The collection includes chemicals of different classes, with variety of functionalities such aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, nitro-derivatives, and multifunctional compounds. *In vivo* and *in vitro* (predominantly, with liver microsomes as experimental systems) studies were used to analyze the metabolic fate of chemicals. Different sources, including monographs, scientific articles and public websites were used to compile the database [12, 15].

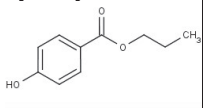
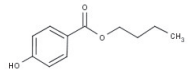
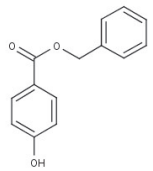
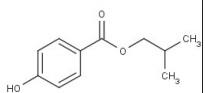
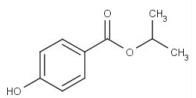
RESULTS AND DISCUSSION

The results of the probable metabolic activation in liver (observed and predicted) and, respectively, protein and DNA binding of some parabens are presented in Table 1.

Electrophilic metabolites may not only react with nucleophilic sites in DNA but may also bind to proteins, RNA, and to endogenous substances of lower molecular weight such as glutathione [13]. The complexity of the reaction of electrophilic metabolites with the various nucleophilic sites within cells and the reasons why different electrophilic reagents react at different sites have been interpreted on the basis of the concepts of hard and soft electrophiles/nucleophiles (hard and soft acids/bases) [21, 23, 27].

Table 1 Possible metabolic activation, DNA and protein binding of some parabens by (Q)SAR Application Toolbox

№	CAS number, Name and structure of parabens	Observed liver metabolism by Toolbox	Liver Metabolism Simulator by Toolbox
1	99-76-3 Methyl-p-hydroxybenzoate 	0 metabolites;	2 metabolites; Protein binding – No binding; DNA binding – No binding;
2	120-47-8 Ethyl-p-hydroxybenzoate 	0 metabolites;	4 metabolites; Protein binding – 3 metabolites are No binding and 1 metabolite – Schiff base formation; DNA binding – No binding;

3	94-13-3 Propyl-p-hydroxybenzoate 	0 metabolites;	4 metabolites; Protein binding – 3 metabolites are No binding and 1 metabolite – Schiff base formation; DNA binding – No binding;
4	94-26-8 Butyl-p-hydroxybenzoate 	0 metabolites;	4 metabolites; Protein binding – 3 metabolites are No binding and 1 metabolite – Schiff base formation; DNA binding – No binding;
5	94-18-8 Benzyl-p-hydroxybenzoate 	0 metabolites;	4 metabolites; Protein binding – No binding; DNA binding – No binding;
6	4247-02-3 2-Methylpropyl 4-hydroxybenzoate 	0 metabolites;	4 metabolites; Protein binding – 3 metabolites are No binding and 1 metabolite – Schiff base formation; DNA binding – No binding;
7	4191-73-5 1-Methylethyl-4-hydroxybenzoate 	0 metabolites;	9 metabolites; Protein binding – 6 metabolites are No binding, 2 metabolites – Schiff base formation and 1 metabolite – Nucleophilic addition to ketones; DNA binding – No binding;

CONCLUSIONS

After uptake into the blood stream, distribution to organs occurs, but due to the rapid hydrolyzation and conjugation, concentrations of parent compounds also are expected to be very low at the active sites in target organs. Thus, it is obvious to consider whether the toxicity of parabens is due to their metabolites, and further studies determining metabolite concentrations in blood and target organs are required.

REFERENCES

[1] Akomeah F., Nazir T., Martin G.P., Brown M.B., "Effect of heat on the percutaneous absorption and skin retention of three model penetrants", European Journal of Pharmaceutical Sciences, 21 (2004) 337–345.

[2] Blair R.M., Fang H., Branham W.S., Hass B.S., Dial S.L., Moland C.L., et al., "The estrogen receptor relative binding affinities of 188 natural and xenochemicals: structural diversity of ligands", Toxicol Sci, 54 (2000) 138–153.

[3] ChemIDPlus website: http://chem.sis.nlm.nih.gov/chemidplus/

[4] Darbre P.D., Aljarrah A., Miller W.R., Coldham N.G., Sauer M.J., Pope G.S., "Concentrations of parabens in human breast tumours", Journal of Applied Toxicology, 24 (2004) 5–13.

[5] Darbre P.D., Byford J.R., Shaw L.E., Hall S., Coldham N.G., Pope G.S., et al., "Oestrogenic activity of benzylparaben", Journal of Applied Toxicology, 23 (2003) 43–51.

[6] Darbre P.D., Byford J.R., Shaw L.E., Horton R.A., Pope G.S., Sauer M.J., "Oestrogenic activity of isobutylparaben in vitro and in vivo", J Appl Toxicol, 22 (2002) 219–226.

[7] Derache R., Gourdon J., "Metabolism of a food preservative: parahydroxybenzoic acid and its esters", Food and Cosmetics Toxicology, 1 (1963) 189–195.

[8] Elder R.L., "Final report on the safety assessment of methylparaben, ethylparaben, propylparaben, and butylparaben", Journal of the American College of Toxicology, 3 (1984) 147–209.

[9] Jackson E.M., "Moisturizers of Today", Journal of Toxicology - Cutaneous and Ocular Toxicology, 11 (1992) 173–184.

[10] Koda T., Umezu T., Kamata R., Morohoshi K., Ohta T., Morita M., "Uterotrophic effects of benzophenone derivatives and a *p*-hydroxybenzoate used in ultraviolet screens", Environmental Research, 98 (2005) 40–45.

[11] Lobemeier C., Tschoetschel C., Westie S., Heymann E., "Hydrolysis of parabenes by extracts from differing layers of human skin", Journal of Biological Chemistry, 377 (1996) 647–651.

[12] Mekenyan O.G., Dimitrov S.D., Pavlov T.S. and Veith G.D., "A systematic approach to simulating metabolism in computational toxicology. I. The TIMES heuristic modeling framework". Current Pharmaceutical Design, 10 (2004) 1273-1293.

[13] Miller J.A., "The metabolism of xenobiotics to reactive electrophiles in chemical carcinogenesis and mutagenesis: a collaboration with Elizabeth Cavert Miller and our associates", Drug Metabolism Reviews, 30 (1998) 645-674.

[14] Mitchell J.R., Jollow D.J., Potter W.Z., et al., "Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione". J. Pharmacol. Exp. Ther., 187 (1973) 211-217.

[15] OECD (Q)SARs Application Toolbox:

[16] Oishi S., "Effects of butylparaben on the male reproductive system in rats", Toxicol Ind Health, 17 (2001) 31–39.

[17] Oishi S., "Effects of butyl paraben on the male reproductive system in mice", Archives of Toxicology, 76 (2002) 423–429.

[18] Oishi S., "Effects of propyl paraben on the male reproductive system", Food and Chemical Toxicology, 40 (2002) 1807–1813.

[19] Oishi S., "Lack of spermatotoxic effects of methyl and ethyl esters of *p*hydroxybenzoic acid in rats", Food and Chemical Toxicology, 42 (2004) 1845–1849.

[20] Okubo T., Yokoyama Y., Kano K., Kano I., "ER-dependent estrogenic activity of parabens assessed by proliferation of human breast cancer MCF-7 cells and expression of ERalpha and PR", Food and Chemical Toxicology, 39 (2001) 1225–1232.

[21] Pearson R.G., Songstad J., "Application of the principle of hard and soft acids and bases to organic chemistry", Journal of American Chemical Society, 89 (1967) 1827-1836.

[22] Pedersen K.L., Pedersen S.N., Christiansen L.B., Korsgaard B., Bjerregaard P., "The preservatives ethyl-, propyl- and butylparaben are oestrogenic in an in vivo fish assay", Pharmacology & Toxicology, 86 (2000) 110–113.

[23] Prescott L.F., "Hepatotoxicity of mild analgesics". Br J Clin Pharmac., 10 (1980) 373-379.

[24] Pugazhendhi D., Pope G.S., Darbre P.D., "Oestrogenic activity of *p*-hydroxybenzoic acid (common metabolite of paraben esters) and methylparaben in human breast cancer cell lines", Journal of Applied Toxicology, 25 (2005) 301–309.

[25] Rastogi S.C., Schouten A., de Kruijf N., Weijland J.W., "Contents of methyl-, ethyl-, propyl-, butyl- and benzylparaben in cosmetic products", Contact Dermatitis, 32 (1995) 28–30.

[26] Routledge E.J., Parker J., Odum J., Ashby J., Sumpter J.P., "Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic", Toxicology and Applied Pharmacology, 153 (1998) 12–19.

[27] Soni M.G., Burdock G.A., Taylor S.L., Greenberg N.A., "Safety assessment of propyl paraben: a review of the published literature", Food and Chemical Toxicology, 39 (2001) 513–532.

[28] Soni M.G., Carabin I.G., Burdock G.A., "Safety assessment of esters of *p*hydroxybenzoic acid (parabens)", Food and Chemical Toxicology, 43 (2005) 985–1015.

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