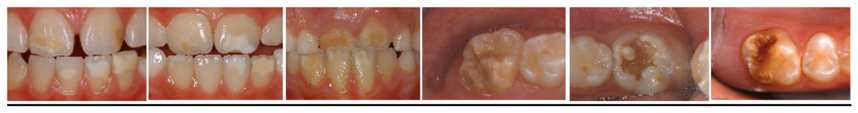
BISPHENOL A AFFECTS AMELOGENESIS BY MODULATING ENAMEL KEY GENE EXPRESSION

Katia Jedeon, Sophia Houari, Blake McAlpin, Sophia Loiodice, Ariane Berdal, Sylvie Babajko
INSERM UMRS 1138, Laboratory of Molecular Oral Pathophysiology - 15 rue de l'école de médecine, 75006 Paris, France.

INTRODUCTION

Bisphenol A (BPA) is a widespread Endocrine Disrupting Chemical (EDC) commonly used by plastic industries as the base compound of polycarbonates and epoxy resins. The consequence of this omni-presence is that more than 95% of the world population contains BPA (ng/ml) in biological fluids raising the question of its activity and potential health adverse effects. Anecdotally, Molar Incisor Hypomineralization (MIH), a recently described enamel pathology affecting 15 to 18% of 6-9 years old children, is increasing concomitantly with EDC related pathologies. Our previous data show that BPA impacts amelogenesis and enamel mineralization and generates similar enamel defects as those described for MIH. The resulting irreversible enamel defects may provide an easily accessible marker for reporting early EDC exposure in humans (1,2).



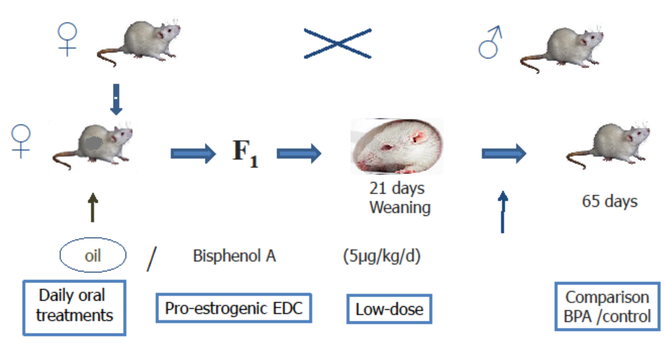
MIH

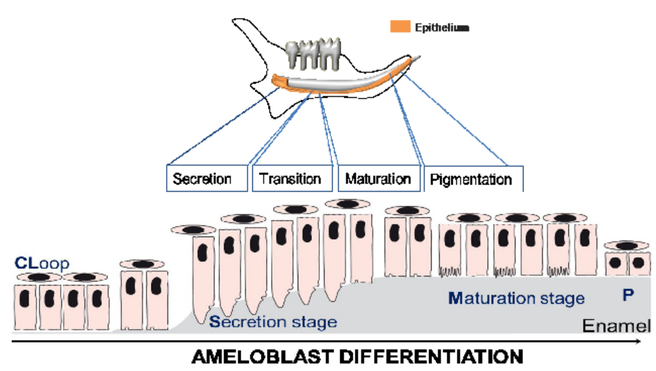
AIM OF THE STUDY

The aim of the present study was to identify BPA target genes involved in amelogenesis in order to decipher the mechanism of action of low-dose BPA in enamel hypomineralization.

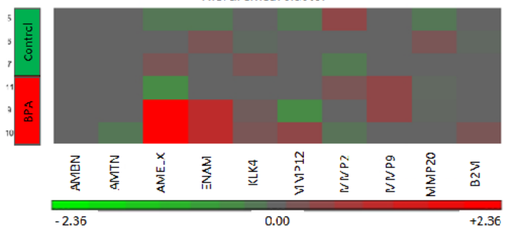
MATERIALS AND METHODS

In vivo, Wistar rats bred in BPA- and phthalate-free conditions were exposed continuously to low-dose BPA (5 µg/kg/day) from the first day of fetal life (E1) to 65 days after birth (P65). RNAs from microdissected dental epithelia were submitted to microarray analysis.
In vitro, analyses were carried out on the rat ameloblastic cell line HAT7 treated by 10^{-9} M BPA.





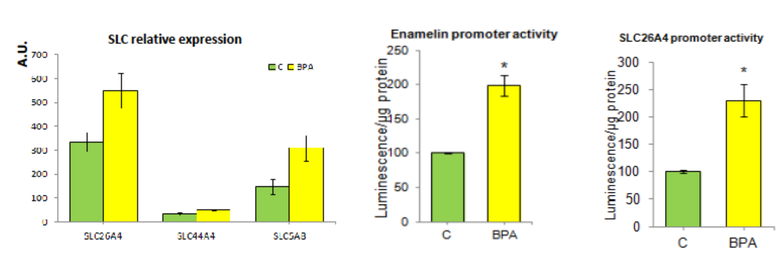
RESULTS



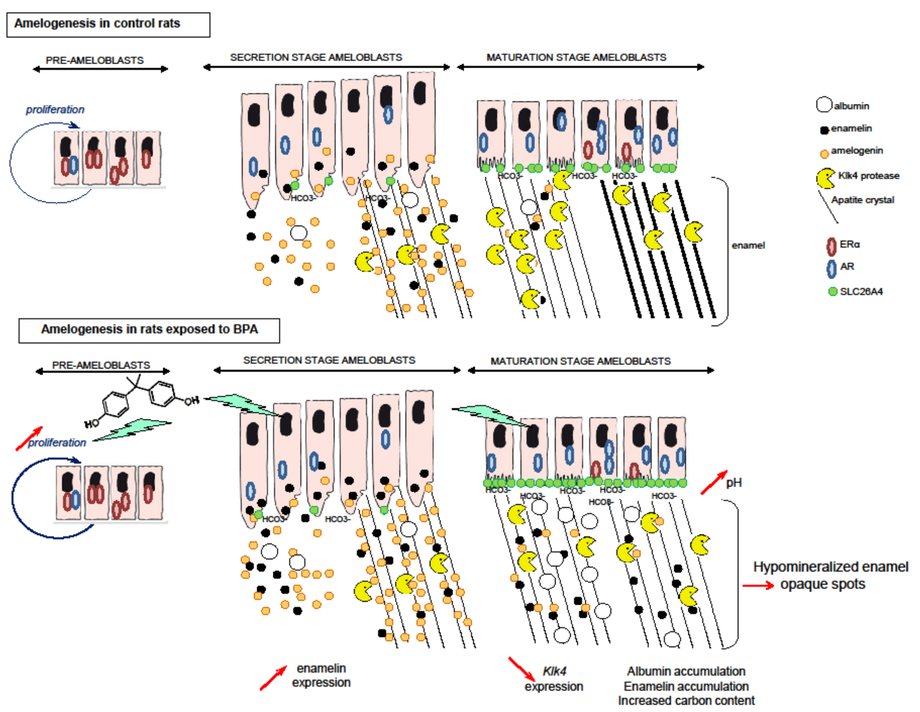
ID	p-value	Fold-Change	Symbol	Gene Name
303924	0.0080	2.90	UPK1B	uroplakin 1B
25295	0.0153	-2.13	BGLAP	bone gamma-carboxyglutamate (gla) protein
360910	0.0344	-2.05	Abcg8l2	ATP-binding cassette, subfamily G (WHITE), member 8-like 2
680963	0.0193	2.03	DUXA	double homeobox A
361619	0.0085	1.83	HBB	hemoglobin, beta
502905	0.0069	-1.77	Klra17 (includes others)	similar to immunoreceptor Ly49si3
500820	0.0177	1.75	**SLC5A8**	solute carrier family 5 (iodide transporter), member 8
171158	0.0075	-1.71	GPIIA2	glycoprotein hormone alpha 2
192649	0.0107	1.69	PROKR2	prokineticin receptor 2
100359743	0.0067	-1.69	SPP1 (includes EG:100359743)	secreted phosphoprotein 1
29704	0.0002	1.69	PACSIN1	protein kinase C and casein kinase substrate in neurons 1
25602	0.0129	-1.68	Tnxa-ps1	tenascin XA, pseudogene 1
100363448	0.0159	1.66	DUXA	double homeobox A
396527	0.0492	1.66	UGT1A3	UDP glucuronosyltransferase 1 family, polypeptide A3
367745	0.0384	1.60	DGKK	diacylglycerol kinase, kappa
100361738	0.0413	1.55	OCIAD2	OCIA domain containing 2
25312	0.0341	-1.54	DMP1	dentin matrix acidic phosphoprotein 1
294255	0.0034	1.51	**SLC44A4**	solute carrier family 44, member 4
25661	0.0424	1.51	FN1 (includes EG:100005469)	fibronectin 1

Column ID	p-value	Fold-Change	Symbol	Gene Name
100360145	0.240501	2.40338	LOC100360145	H2A histone family, member Z-like
302669	0.0511076	2.05585	G6PB	glucose-6-phosphatase, catalytic subunit 2
29160	**0.270352**	**1.86569**	**AMELX**	**amelogenin, X-linked**
295369	0.257751	-1.79356	TNNC2	troponin C type 2 (fast)
297992	0.056618	1.76988	ATP6V0D2	ATPase, H+ transporting, lysosomal 38kDa, V0 subunit d2
503015	0.0591935	-1.75406	SAMD9L	sterile alpha motif domain containing 9-like
64640	0.214400	-1.74236	Gm6570	predicted gene 6570
688354	0.0542204	1.70225	MAB21L1	mab-21-like 1 (C. elegans)
25145	0.140967	1.67703	Cd24a	CD24a antigen
498356	0.157395	1.6332	SHISA3	shisa homolog 3 (Xenopus laevis)
498855	0.105065	1.6331	C20orf54	chromosome 20 open reading frame 54
24584	0.383745	1.50593	MYLPF	myosin light chain, phosphorylatable, fast skeletal muscle
280921	0.0888509	1.00154	AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)
287699	0.237037	-1.59403	KRT13	keratin 13
287700	0.164037	1.58991	KRT15	keratin 15
362268	0.114537	1.58261	RTEL1	regulator of telomere elongation helicase 1
29440	0.0755258	1.57538	**SLC26A4**	solute carrier family 26, member 4
363205	0.124653	1.57444	RGD1562107	similar to class-alpha glutathione S-transferase
25163	0.0787418	1.55858	CDKN2A	cyclin-dependent kinase inhibitor 2A
100361866	0.0872892	1.55524	Ear5	eosinophil-associated, ribonuclease A family, member 5
280525	**0.190034**	**1.50036**	**ENAM**	**enamelin**
501816	0.179228	1.50495	Vcan2r120	versican-like 2, transcript 120

Among 19239 RNAs, only 19 RNA levels were significantly modulated (more than 1.5-fold) by BPA. As 75% of exposed rats were responsive to BPA (1), it was justified to select the 41 genes modulated more than 1.5-fold after exposure to BPA even if not significantly. Among these genes, amelogenin and enamelin coding for specific enamel matrix proteins were ones of the highest genes up-regulated, 1.87-fold and 1.51-fold respectively. SLC26A4 which encodes an anion exchanger involved in mineralization process also appeared as a target gene. SLC26A4 could be involved in pH regulation by secreting bicarbonates to neutralize protons released into the enamel space during crystal growth (3). SLC5A8 and SLC44A4 are also solute carriers which expression was induced by BPA 1.51- and 1.75-fold respectively. However, they are 2- and 10-fold less expressed than SLC26A4.



Luciferase reporter assays evidenced transcriptional modulations of enamelin. Further studies are currently underway to decipher transcriptional modulations of SLC26A4 in relation with steroid receptors (ERα and AR) already shown to be involved in BPA effects during amelogenesis.



CONCLUSION

In conclusion, we report that BPA impacts ameloblast differentiation and enamel synthesis through modulations of enamel key gene expression. Despite the small number of BPA target genes specifically expressed in ameloblasts, factors that transmit BPA effects are ubiquitous and involved in general pathophysiology. Thus, these data help to understand how enamel defects may be used as early marker of exposure to EDCs that act as BPA.

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Jedeon Katia
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