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Infants' exposure to aluminum from vaccines and breast milk during the first 6 months

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The success of vaccination programs in reducing and eliminating infectious diseases has contributed to an ever-increasing number of vaccines given at earlier ages (newborns and infants). Exposure to low levels of environmental toxic substances (including metals) at an early age raises plausible concerns over increasingly lower neuro-cognitive rates. Current immunization schedules with vaccines containing aluminum (as adjuvant) are given to infants, but thimerosal (as preservative) is found mostly in vaccines used in non-industrialized countries. Exclusively, breastfed infants (in Brazil) receiving a full recommended schedule of immunizations showed an exceedingly high exposure of Al (225 to 1750 μg per dose) when compared with estimated levels absorbed from breast milk (2.0 μg). This study does not dispute the safety of vaccines but reinforces the need to study long-term effects of early exposure to neuro-toxic substances on the developing brain. Pragmatic vaccine safety needs to embrace conventional toxicology, addressing especial characteristics of unborn fetuses, neonates and infants exposed to low levels of aluminum, and ethylmercury traditionally considered innocuous to the central nervous system.
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Introduction

Although vaccine safety is constantly reaffirmed in regard to its immunogenicity and rare adverse events, it is assumed that low doses of preservative (thimerosal) and adjuvant (aluminum salts) have the same innocuous effects across the large spectrum of those vaccinated — adults, children, infants, newborns, and unborn fetuses — and for the ever-increasing number of them given to young children. Despite low doses in vaccines, both Hg and Al are neuro-toxic; the higher toxicity of Hg is well recognized and it has been more studied and better understood than Al.

During early life, exposure to either mercury or aluminum that occur through breastfeeding depends on the maternal exposure (diet mainly). However, because of mammary-gland barrier, expected exposure for infants is greatly attenuated. The exposure to mercury or aluminum in breast milk is spread out through the course of a day's nursing with the very young or smaller (immature) baby absorbing proportionally smaller quantities. However, in intramuscular injections ethylmercury (in preservatives) and Al (as adjuvant) gain unimpeded access to body compartments. In this context, specific aspects of Hg exposure have been discussed elsewhere (Dórea, 2007). The American Academy of Pediatrics' revision of 1996 discussed aluminum in infant feeding but did not address the additional higher and acute exposure to aluminum in commonly used infants' vaccines (AAP, 1996).

Recent evidence based on cellular and animal studies indicates that both thimerosal at small concentrations (Baskin et al., 2003; Hornig et al., 2004; Ueha-Ishibashi et al., 2004; James et al., 2005; Parran et al., 2005; Geier et al., 2009; Hewitson et al., 2009; Olczak et al., 2009) and adjuvant-Al are neuro-toxic. In this regard, aluminum-adsorbed vaccines caused a transient rise in brain tissue of mice (Redhead et al., 1992). Indeed, *in vitro* work showed that adjuvant-Al at levels comparable to those administered to adults can kill motor neurons (Petrik et al., 2007). Toimela and Tähti (2004) showed the toxicity of both Al and Hg in neuro-blastoma cell line. The toxicity of Al is much lower than that of thimerosal (Deth et al., 2008). Nevertheless, Mutter et al. (2007) suggested that low levels of Hg could cause nerve cell deteriorations that could be aggravated by aluminum. Therefore, data to provide a non-observable adverse effect level for Hg and Al (inclusive combined) on the brain are sorely needed.

Vaccines represent an important strategic line of defense against infectious diseases; however, those containing mercurial

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preservatives and aluminum adjuvants raise issues of early life exposure to low levels of neuro-toxic substances. This study shows the need to address low-dose exposure to combined Al and Hg as an issue (infant's neuro-cognitive development) beyond acute events notified as vaccine adverse effects.

Patients and methods

The vaccine information used in this study has been adapted from previous publications (Marques et al., 2007a). The research protocol of the original study was approved by the ethics committee of studies for humans of the Universidade Federal de Rondonia and details appeared elsewhere (Marques et al., 2007a). Briefly, the study was designed to evaluate the role of fish consumption in growth and development of children. During the process of publishing the first paper, we became aware of the presence of thimerosal used to preserve vaccines. After our involvement with studies of ethylmercury exposure through thimerosal-containing vaccines (TCV) (Marques et al., 2007b, c, d, 2008, 2009) we became aware that another neuro-toxic element — aluminum — is also used in the same TCV as adjuvant.

The infants in this study received a dose of hepatitis B (HB) vaccine before discharge from the maternity ward; mothers followed the immunization schedule recommended by the Ministry of Health of Brazil and returned at 30, 60, 120, and 180 days when thimerosal vaccines (HB and diphtheria, tetanus, and pertussis (DTP)) were inoculated.

Estimated Exposure to Aluminum from Vaccines and Breastfeeding

The Hg concentration of the doses delivered through vaccines was respectively 12.5 μg/0. 5 ml and 25 μg/0.5 ml for HB and DTP; as stated by manufacturers, HB has 0.01% Thimerosal/dose (Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, 0.01% Thimerosal (LG Life Sciences, Jeonbuk-Do, Korea); Vacina Recombinante, 10 mg Thimerosal/dose (Instituto Butanta, São Paulo, Brazil; 12.5 μg Thimerosal/dose ENGERIX-B (Hepatitis B Vaccine (Recombinant)) Smith Kline Beecham Biologicals, Rixensart, Belgium) and DTP has also 0.01% Thimerosal/dose (Triple Antigen, Serum Institute of India, India; Vacina Tríplice, (Instituto Butanta, São Paulo, Brazil)). The aluminum (adjuvant) concentrations of these vaccines were respectively 250 μg/0.5 ml and 1500 μg/0.5 ml for HB and DTP.

Differences in infants' weight at birth and at 6 months were used to estimate daily weight gain and integrated gain at 30, 60, 120, and 180 days. Exposure to Hg and Al through breast milk was estimated as described before (Marques et al., 2007b): infant mean weight $\times$ mean daily breast milk consumption (140 ml/kg) $\times$ number of days $\times$ mean total metal concentrations. For Hg concentration in breast milk (1.9 μg/l) we used the median value reported by Dórea (2004), whereas for Al concentration we used 40 μg/l from conventional references adopted by others (AAP, 1996; Keith et al., 2002; Offit and Jew, 2003) and the maximum absorption of 0.1%.

Results

The exposure to Al during the first 6 months is shown in Table 1. Exposure to Al from adjuvant used in vaccines was calculated from information provided by the vaccine manufacturers, while exposure from breastfeeding was estimated from data available in the literature; as it is, there are no data on Al concentrations in milk of Brazilian women. All infants' vaccines that contain aluminum as adjuvant also contain thimerosal as preservative. Therefore, because of possible interactive effects, we also listed the exposure figures for total Hg derived from vaccines and breastfeeding.

Table 1. Infant immunization schedule, type of vaccine, and respective dose of ethyl-Hg (etHg) and Al; estimated Al intake from breast milk at time of vaccination is also shown as illustration.

Age, d	Body weight, kg	Vaccine, μg metal/dose			Breast milk[b] Al intake, μg
		Type[a]	etHg	Al	Al
0	3.23	HB	12.5	250	250	0
30	3.86	HB	12.5	250	250	22.6
60	4.49	DTP	25.0	1250	1500	26.3
90	5.06	—				
120	5.75	DTP	25.0	1250	1500	33.58
150	6.32	—				
180	7.01	DTP + HB	37.5	1500	1750	40.9

[a]HB: Hepatitis B (assumed 0.01% Thimerosal/dose and 250 μgAl/dose, Korea Green Cross Corporation, Kiheung-Eup Yougin-Goon Kiyunggi-Do, Korea; Euvax B injectable, 0.01%Thimerosal and 250 μgAl/dose (LG Life Sciences, Jeonbuk-do, Korea)); DTP (Serum Institute of India, 1,500 μgAl/dose; Vacina Tríplice, 0.01% Thimerosal/dose and 1,250 μgAl/dose (Instituto Butanta, São Paulo, Brazil)).
[b]Integrated total Hg intake (estimated from Dórea, 2004).

The striking difference between adjuvant-Al (non-enteral) and estimated breast-milk-Al (enteral) is illustrated in Figure 1 on a body mass basis. Newborns (at day 0) not yet breastfeeding (and not passing stools) are exposed to aluminum exclusively from the HB vaccine. The amount of Al received from the vaccines' adjuvant is far greater than small amounts (0.01%) that can be derived from breast milk (Figure 1); the magnitude of the acute dose of the two toxic metals during the series of immunizations depends on type of vaccine and the manufacturer. As an illustration, the first jab of HB with the lowest Al dose (250 μg) is five times the total exposure of absorbed Al (55 μg) through the next 6 months of breastfeeding.

Discussion

This study reveals that iatrogenic exposure to acute dose of aluminum occurs in newborns and in infants at greater amounts than through breastfeeding. The estimated amount of Al available after absorption from breastfeeding contrasts with the non-enteral high adjuvant-Al doses in serial vaccination schedules. Environmental aluminum through breastfeeding, as a consequence of dietary intake of mothers, encounters several physiological barriers (maternal gut → mammary-gland → infant gut). These barriers are absent in non-enteral adjuvant-Al exposure; the high acute doses of adjuvant-Al (250 to 1,500 μg) constitute a neurological challenge to neonates and are never encountered by young humans even when exposed to high Al infant formulas.

It is often noted that infants are exposed to Al in breast milk (40 μg/l) and in infant formulas at levels of 225 μg/l and that this approaches Al concentrations of some vaccines (Keith et al., 2002; Offit and Jew, 2003). Although the half-life of enterally absorbed Al elimination from the body is short (approximately 24 h), the same cannot be assumed for adjuvant-Al; because of "depot effect" a longer elimination is one of the very functions of adjuvants. Indeed a tightness of bonding between the aluminum adjuvant and the antigen is a desired feature that can be used to predict immunogenicity of vaccines (Egan et al., 2009).

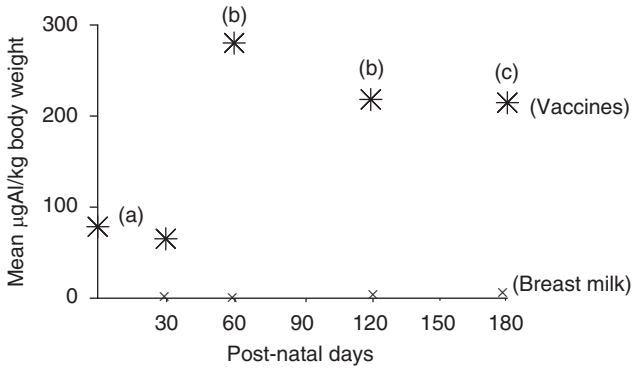

Figure 1. Aluminum body load injected from vaccines and estimated from breast-milk intake; (a) Hepatitis B (HB), (b) DTP, (c) HB + DTP. DTP vaccines' aluminum concentrations of 1,500 μg/dose are not represented (values are respectively 333.3, 263.1, 250 μg/kg/b.w.). Estimated Al available (0.1%) from breastfeeding were respectively 0.22, 0.25, 0.32, 0.39 μg/kg/b.w.

The HB vaccine has both Hg and Al at lowest concentration. However, given the special characteristics of the first dose, the variability in dose can be substantial. Recently, we showed that on a body mass basis neonates can have a wide variation in the dose of thimerosal-Hg (from 2.1 to 21.1 μg/kg) depending on the vaccine manufacturer (Dórea et al., 2009); this amplitude in variability is extended to aluminum. Therefore, Keith et al. (2002)'s model of first-year body burden of Al (from vaccines and feeding) is insufficient to show low risk of brain-Al derived from acute high exposure to adjuvant-Al in vaccinated neonates.

Furthermore, the 1-day neonate has anatomical and functional differences crucial for toxicokinetis and toxicodynamics of neuro-toxic metals: an immature renal system and a developing blood-brain barrier; these and other modifying circumstances can be aggravated by shorter gestational age, pre-maturity, or low birth weight (Dórea et al., 2009). In addition, neonates (<24 h and $>2{,}000$ g) may receive a dose of adjuvant-Al (250 μg) in HB vaccine that is equivalent to a >6 month exposure to absorbed Al in breast milk. Therefore, safety assumptions derived from feeding (high quantities) of aluminum to experimental animals may not be an appropriate proxy for non-enteral exposure (frequently in combination with thimerosal-Hg) in neonates as it is currently accepted (Offit and Jew, 2003).

In relation to vaccine adverse events, the track record established in the past 70 years is a convincing argument for the safety of adjuvant-Al (Offit and Jew, 2003) for non-susceptible individuals. However, neuro-behavioral, cognitive and learning impairment effects of small doses of toxic metals took place in the last 40 years; neuro-developmental studies of toxic metals as preservatives and adjuvants in vaccines are starting to appear (Marques et al., 2009). A report by Gallagher and Goodman (2008) suggested an association of HB vaccines and a higher risk of receiving special education services. Although HB vaccines have aluminum hydroxide as adjuvant (Baylor et al., 2002) the paper by Gallagher and Goodman (2008) draws attention only to thimerosal-Hg.

Mild post-vaccine symptoms in young infants, especially neonates, are non-specific and considered tolerable; rare (neurologic) adverse effects are unlikely to occur as a result of adjuvant-Al *per se* or in combination with thimerosal-Hg. As a descriptive study it is only possible to make clear that future research is needed to ascertain neuro-toxic effects and risks of non-enteral binary exposure to preservatives and adjuvants in vaccines destined for young infants. To date, we have no clue as to the effects of combined ethylmercury and aluminum

dose unlikely to be encountered through environmental exposure in breastfed (or even formula fed babies) and as such do not understand its low-dose effect on brain function regarding cognitive and learning impairments occurring later.

Conclusion

At present, because of the universal coverage of vaccines, the child population can be exposed to a combination of Hg and Al at very early ages. Therefore, it is critical for increasing trust in vaccination that we understand the nature, intensity, and plausible neuro-behavioral consequences of this type of exposure.

Conflict of interest

The authors declare no conflict of interest.

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