Pharmacokinetic modeling as an approach to assessing the safety of residual formaldehyde in infant vaccines

Robert J. Mitkus a,*, Maureen A. Hess b, Sorell L. Schwartz a,c

a Office of Biostatistics and Epidemiology, USFDA Center for Biologies Evaluation and Research, 1401 Rockville Pike, HFM-210, Rockville, MD 20852, United States
b Office of Vaccines Research and Review, USFDA Center for Biologies Evaluation and Research, 1401 Rockville Pike, HFM-405, Rockville, MD 20852, United States
c Department of Pharmacology and Physiology, Georgetown University Medical Center, 3900 Reservoir Rd NW, Washington, DC 20057, United States

ARTICLE INFO

Article history:
Received 25 July 2012
Received in revised form 5 March 2013
Accepted 31 March 2013
Available online 11 April 2013

Keywords:
Formaldehyde
Inactivating agent
Safety
Pharmacokinetics
Modeling

ABSTRACT

Formaldehyde is a one-carbon, highly water-soluble aldehyde that is used in certain vaccines to inactivate viruses and to detoxify bacterial toxins. As part of the manufacturing process, some residual formaldehyde can remain behind in vaccines at levels less than or equal to 0.02%. Environmental and occupational exposure, principally by inhalation, is a continuing risk assessment focus for formaldehyde. However, exposure to formaldehyde via vaccine administration is qualitatively and quantitatively different from environmental or occupational settings and calls for a different perspective and approach to risk assessment. As part of a rigorous and ongoing process of evaluating the safety of biological products throughout their lifecycle at the FDA, we performed an assessment of formaldehyde in infant vaccines, in which estimates of the concentrations of formaldehyde in blood and total body water following exposure to formaldehyde-containing vaccines at a single medical visit were compared with endogenous background levels of formaldehyde in a model 2-month-old infant. Formaldehyde levels were estimated using a physiologically-based pharmacokinetic (PBPK) model of formaldehyde disposition following intramuscular (IM) injection. Model results indicated that following a single dose of 200 μg, formaldehyde is essentially completely removed from the site of injection within 30 min. Assuming metabolism at the site of injection only, peak concentrations of formaldehyde in blood/total body water were estimated to be 22 μg/L, which is equivalent to a body burden of 66 μg or <1% of the endogenous level of formaldehyde. Predicted levels in the lymphatics were even lower. Assuming no adverse effects from endogenous formaldehyde, which exists in blood and extravascular water at background concentrations of 0.1 mM, we conclude that residual, exogenously applied formaldehyde continues to be safe following incidental exposures from infant vaccines.

Published by Elsevier Ltd.

1. Introduction

Formaldehyde is an effective cross-linking agent that is used in certain vaccines to inactivate viruses and to detoxify bacterial toxins while not materially affecting antigenicity [1]. Formaldehyde may also contribute to the preservation of these vaccines and help ensure that there is no reversion of the biological components back to an active or toxic state [2,3]. As part of the manufacturing process, residual free formaldehyde can remain behind at levels of 0.4–100 μg per 0.5 mL (0.00008–0.02%), depending on the vaccine product; 2.5 μg per 0.5 mL dose (0.0005%) is a typical level of residual formaldehyde measured in some yearly influenza vaccines by FDA chemists [Alfred Del-Grosso, personal communication]. Formaldehyde has not been associated with local or systemic adverse effects following vaccine administration other than a single case of exacerbation of eczema reported in an adult healthcare worker who received a formalin-containing hepatitis B vaccine [4–6].

In humans and other mammals, formaldehyde is produced normally in all cells of the body by oxidative N-demethylation of endogenous metabolic intermediates [7,8]. Accordingly, formaldehyde exists naturally at relatively constant levels of about 0.1 mM in both blood and extravascular tissues, and almost completely in its hydrated form, methanediol, given the predominantly aqueous nature of those compartments [9–11,14,54,55]. Predictably, with a water solubility of 400g/L, exogenous formaldehyde, like methanol, should distribute in total body water, i.e. in a volume of distribution of about 0.7L/kg, and the plasma half-life of exogenously administered formaldehyde is extremely short, approximately 1.5 min [12,13,36]. Assuming a volume of distribution of approximately 50L for a 70-kg individual, this implies a total body formaldehyde turnover rate of approximately 69 mg per minute, based on body water distribution.

* Corresponding author. Tel.: +1 301 827 6083.
E-mail address: Robert.Mitkus@fda.hhs.gov (R.J. Mitkus).

0264-410X/– see front matter. Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.vaccine.2013.03.071
Both endogenous and exogenous formaldehyde are scavenged by glutathione (GSH) and metabolized in a two-step reaction (Fig. 1) involving formaldehyde dehydrogenase (FDH) and S-formylglutathione hydrolase [17]. Formaldehyde may also be metabolized by aldehyde dehydrogenase 1A1 and 2, or catalase; however, FDH, with an ~200-fold lower Michaelis–Menten constant, $K_m$, is the predominant metabolizing enzyme [15,16]. Studies on the distribution of FDH and S-formylglutathione hydrolase in rats and humans indicate that both are present ubiquitously in tissues and at similar levels or activity across tissue [15,18–22]. This reflects the tight homeostatic regulation of formaldehyde in mammalian tissues, especially muscle, blood, and blood vessels [23–25]. Studies have failed to demonstrate significant differences in FDH expression in the human population [17,26,27].

Environmental and occupational exposure to formaldehyde, principally by inhalation, has been and is a continuing risk assessment focus and area of debate and research [10,28,29,65]. This arises from the high reactivity of formaldehyde with small and large biological molecules, as well as from animal and epidemiological data indicating that formaldehyde is a human carcinogen by that route. Exposure to formaldehyde via vaccine administration, however, is qualitatively and quantitatively different from environmental and occupational settings and calls for a different perspective and approach to risk assessment.

There are stark differences in exposure amounts and duration, as well as time factors in absorption and distribution, that distinguish vaccine-source formaldehyde from occupational and environmental sources of exogenous formaldehyde. For example, exposure to formaldehyde in vaccines is parenteral, acute, and infrequent, with a dosing interval in infants of one month or more, compared to occupational exposures which are often daily via the inhalation route, continuous throughout a working day, or chronic. These differences, combined with the understanding that formaldehyde is an exogenous molecule that exists in the body at high background levels, imply that risk assessments relevant to occupational and environmental formaldehyde exposures are not applicable to exposure by the vaccine route. Furthermore, while regulatory limits for both oral and inhalational exposures exist for formaldehyde, those thresholds are primarily for repeat long-term, rather than single-dose, exposures [28,56,57]. Given the significant uncertainty that results from route-to-route as well as intermediate-/long-term to single-dose extrapolations based on those regulatory values, they are not considered relevant for assessing risk from episodic exposures to residual formaldehyde in vaccines.

While several studies have been published in either animals following experimental treatment or in humans following iatrogenic exposures to formaldehyde by the intravenous, intraperitoneal, or intradental route [58–62], we found no published data on the fate of intramuscular formaldehyde in any species of any age. In the absence of relevant experimental or clinical data on levels of formaldehyde in the body via the IM route, we developed a physiologically-based pharmacokinetic (PBPK) model that estimates both local and systemic levels of vaccine formaldehyde following IM administration. We then compared our model estimates with known steady-state levels of endogenous formaldehyde and utilized that comparison as the basis of an assessment of the safety of formaldehyde in infant vaccines. Our assessment is based on a plausibility argument: it is neither deductive, wherein the conclusion logically derives from the premise; nor inductive, wherein the conclusion is probabilistically derived from the premise. Plausibility has been subsumed, in some descriptions, under abduction; and Walton cites Rascher in stating that “[P]lausibility . . . evaluates propositions in relation to the standing and solidity of their cognitive basis by weighing available alternatives” [37]. It is beyond the scope of this assessment, however, to proffer a complete exposition of the philosophical underpinnings of plausibility arguments.

2. Methods

2.1. Pharmacokinetic modeling

The model used for pharmacokinetic estimations was based on a hypothetical 2-month-old, 4.2 kg infant who, by these parameters is in the lower 10th percentile (United States) for age-weight relationship [30]. This age was chosen because a low-birth-weight,
2-month-old infant could receive the highest maximum exposure to formaldehyde-containing vaccines per unit body weight. Specifically, this would obtain in the 4.2-kg infant receiving 208 μg formaldehyde from Tripeida® (DTaP, diphtheria tetanus and acellular pertussis), ActHIB® (Hib, Haemophilus B conjugate), IPOL® (IPV, inactivated polio), and Recombivax HB® (HepB, hepatitis B) products at a single office visit, according to the 2012 vaccination schedule published by the Advisory Committee on Immunization Practices of the Centers for Disease Control and Prevention [31,52]. A 6-month-old infant could receive a higher combined dose of formaldehyde (~250 μg) from the four vaccines mentioned above plus 50 μg from the Fluzone® influenza vaccine at a single office visit; however, since a 6-month-old weighing 6.8 kg (lower 10th percentile) is larger than a 2-month-old, s/he would actually receive a lower dose per unit body weight. In addition, because some healthcare providers may use alternative immunization schedules and some vaccine products contain lower amounts of formaldehyde than others, it is unclear how typical either of those formaldehyde exposures would be. For example, Pediatrix®, a combined DTaP/IPV/HepB vaccine that an infant might receive at 2 months of age, contains less than or equal to 100 μg formaldehyde per dose. Therefore, we assumed a maximum single dose of 200 μg as an upper estimate of formaldehyde exposure in our model.

The anatomical and physiological parameter values for our model are summarized in Table 1 and were derived as follows: total body water, 0.71-L·kg\(^{-1}\) [32]; blood volume, 80 mL·kg\(^{-1}\) [32]; quadriceps femoris muscle volume, 12 mL·kg\(^{-1}\), which was based on adult volume [33] corrected for infant/adult body composition difference (0.55) and infant/adult muscle distribution between the upper and lower extremities (0.55) [32]; cardiac output, 235 mL·min\(^{-1}\)·kg\(^{-1}\bw\) [34]; quadriceps blood flow, 38 mL·min\(^{-1}\)·kg (tissue)\(^{-1}\) [35]; \(K_m\) of FDH in rat respiratory/olfactory tissue, 0.09 μg/mL (based on [16], in absence of a value in humans); \(V_{\text{max}}\) of FDH in muscle, 189 μg/min (derived from [22]). A physiologically-based pharmacokinetic model was developed that related the quadriceps muscle to rest of the body (Fig. 2).

The quadriceps muscle was divided into two zones, an administration zone (qf1) and a metabolic zone (qf2). This was to account for the time delay in formaldehyde distribution among the muscle fibers and intracellular entry. The proportion of space allotted to the qf1 zone was an estimate, the accuracy of which is not consequential, as is discussed below. Estimates of formaldehyde in blood and total body water and at the site of injection post dose were made using the physiologically-based pharmacokinetic (PBPK) modeling software, CMATRIX [53]. Distribution into these media following injection was based on its very high water solubility (400 g/L [36]). The purpose of the PBPK model was also to focus on administered formaldehyde as it is distributed within and leaves the muscle, physically or metabolically. Consequently (as a nod to the principle of parsimony – Occam’s razor), extra-muscle metabolism was not included in the model, although it can be assumed to occur given the ubiquitous presence of FDH in mammalian tissues.

### Table 1: Physiological inputs for the PBPK model of formaldehyde disposition in infants.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Volume (ml)</th>
<th>Blood flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>330</td>
<td>987 (cardiac output)</td>
</tr>
<tr>
<td>Body water reservoir</td>
<td>2670</td>
<td>985.5</td>
</tr>
<tr>
<td>Quadriceps zone 1</td>
<td>545</td>
<td>1.9 (total)</td>
</tr>
<tr>
<td>Quadriceps zone 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Results and discussion

As a first, and understandably rudimentary, approximation, a classical one-compartment pharmacokinetic model was employed. Irrespective of cross-link formation, the endogenous formaldehyde blood concentration of 0.1 mM reflects the concentration in total body water, almost exclusively as methanediol, save for corrections for cellular and protein blood components. The total body water in the model infant is 3 L, so that the total endogenous formaldehyde in body water is 9 mg (30 mg·mmol\(^{-1}\)\times 0.1 mM \times 3 L). Continuing the starting-point analysis, the administration of 200 μg of formaldehyde would increase the total body water content of formaldehyde by approximately 2%, or 2 μM.

Clearly, the estimate of formaldehyde concentration in total body water after a one-compartment distribution is inaccurate; it ignores the metabolism of formaldehyde and its distribution from an intramuscular site. Therefore it is an overestimate of additional

---

Fig. 2. PBPK model of formaldehyde disposition in infants (a) and differential equations (b). Model differential equations were generated and solved using the software CMATRIX. Q\(_{\text{blood}}\): arterial blood concentration; C\(_{\text{compart}}\): physiological compartment concentration, which, in this model, is equal to the effluent venous blood concentration; Q\(_{\text{incompart}}\): physiological compartment blood flow; V\(_{\text{compart}}\): physiological compartment volume; \(K_{1:2}\): diffusion constant between qf1 and qf2; \(V_{\text{max}}\) and \(K_m\): Michaelis–Menten kinetics parameters.
distributed formaldehyde provided by the 200 μg dose. The essential question is whether or not the administered formaldehyde gains access to tissue sites distinct from endogenous formaldehyde. This was assessed by using the physiologically-based pharmacokinetic model shown in Fig. 2a and b.

Fig. 3 shows the blood and body reservoir formaldehyde concentrations and total muscle formaldehyde content over the 30-min post administration period based on our PBPK model. The peak body water concentration of formaldehyde in the model infant is just under 22 μg/L following a 200 μg dose. This is equivalent to a body burden of approximately 66 μg formaldehyde, or <1% of the endogenous level of formaldehyde, which we consider more plausible than the 2% estimate from the one-compartment model. This body burden would have been even lower had we included the contribution of extra-muscular metabolism by the ubiquitous enzyme, formaldehyde dehydrogenase, to the elimination of formaldehyde in the model. Model results also indicate that formaldehyde is almost completely removed from muscle within 30 min of a single dose.

We started our analysis with the premise that the vaccine-related exogenous formaldehyde is pharmacokinetically indistinct from endogenous formaldehyde, and, as mentioned, determined that it is not. It is initially concentrated in a section of muscle. It leaves the site of injection by two well-known means that are represented in our PBPK model: (1) circulating blood and (2) metabolism. There is another possible route of departure from the muscle that is not represented in the PBPK model, i.e. entry into lymphatics. Lymph production is the means of maintaining an iso-volumetric and iso-gravimetric interstitial space. Conceivably, an intramuscular injection could increase interstitial fluid hydrostatic pressure, resulting in bulk fluid movement from the muscle lymphatic capillaries. The flux of water would carry with it the molecules in that water, including formaldehyde. Lymphatic flow is reported to be 50–100 times less than blood flow [38]. Using a PBPK model wherein a 0.5 mL lymph compartment was included in the quadriceps muscle, and in direct contact with qf zone 1 (Fig. 2a), and a lymph flow of 0.04 mL/min, it is estimated that, if any formaldehyde at all leaves the muscle via lymph drainage, it is not more than 0.3% of the dose. This loss would also occur within 30 min after dose administration. This is consistent with the findings by Navas et al. [39] on the removal of intramuscularly administered 99mTc-labeled albumin from at-rest muscle via lymphatic flow, i.e. 0.06 ± 0.05% min⁻¹, and taking into account that unlike formaldehyde, there is no muscular metabolism of the injected albumin. Accordingly, insignificant formaldehyde content is added to the lymph, when compared with the endogenous concentration (equal to body water concentration). Specifically, endogenous formaldehyde levels (9 mg) are likely to be at least four orders of magnitude higher than any exogenous formaldehyde that might “escape” into the lymphatics following a 200 μg dose of formaldehyde.

We do not expect adverse effects from vaccine formaldehyde for several reasons. First, the background levels of formaldehyde in an infant are 136 × (9000 μg/66 μg) higher than peak systemic levels of formaldehyde following vaccine administration. To expect an adverse effect from such a small amount of exogenous formaldehyde would necessarily imply adverse effects from endogenous formaldehyde, which is present at much higher levels. We could find no experimental or clinical evidence in the biomedical literature that demonstrated any adverse effect from endogenous formaldehyde. In fact, human cells are exposed to ~1000 endogenous formaldehyde-DNA adducts, among many other forms of DNA damage, at any given time [7,43]. Importantly, glutathione (GSH) and other electrophile and free radical scavengers, metabolizing enzymes, and DNA repair mechanisms work to keep the levels of reactive endogenous compounds and their adducts under tight control.
homeostatic control [45–47,50]. Lutz [44] has shown that even when endogenous hepatic formaldehyde production was pushed to maximal levels in rats following single oral exposures to an extremely high dose of methanol (1000 mg/kg bw), there was no corresponding increase in DNA–protein crosslinks.

Second, as described above, our PBPK model indicates that a maximum of only a third of the dose (66 µg) will be distributed systemically following a 200-µg dose of exogenous formaldehyde; the remainder would be expected to be dispatched rapidly at the site of injection through GSH scavenging and metabolism (primarily) and tissue binding [40]. Considering the normal range of formaldehyde in tissues related to its role in glycine, methionine, choline, and serine metabolism, and in nucleic acid synthesis, we do not consider it plausible that a <1% change (66 µg/9000 µg) in the systemic level of formaldehyde would create a disease condition where none existed prior to the change [41,42]. Such an effect would imply endogenous formaldehyde under homeostatic control having heretofore unknown sensitivity, one more finely tuned than that for other endogenous compounds including calcium, sodium, and potassium, to name a few. Had we included the activity of formaldehyde dehydrogenase in blood and tissues distant to the site of injection, our estimate of systemically distributed formaldehyde would have been even lower.

We have also considered the possibility that exogenous formaldehyde levels might be “additive to background” (i.e., endogenous) levels and lead to systemic toxicity. We find this possibility to be untenable, since the additivity to background hypothesis assumes an adverse effect from endogenous levels of formaldehyde to begin with [48]; as mentioned earlier, there is no evidence for any adverse effect from endogenous levels of formaldehyde, including tumors. Moreover, we found no evidence in the published literature of an association between vaccination or formaldehyde-containing vaccines and childhood skeletal muscle tumors (rhabdomyoma or rhabdomyosarcoma), both of which are rare in children and adolescents [63,64]. To summarize using language from the National Academy of Sciences (2009) in their most recent guidance document on risk assessment practice, therefore, we would expect no “chemical additivity” [48] of vaccine formaldehyde to endogenous levels of formaldehyde, since, as our model demonstrates, the level of formaldehyde in the body at a single vaccination is a tiny fraction (<1%) of the endogenous level and so short-lived in plasma (t1/2 = 1.5 min) as to be inconsequential. And we would also expect no “biologic additivity” to background [48], since there are no known systemic toxicities in the general population associated with endogenous formaldehyde to which vanishingly small amounts of vaccine formaldehyde could contribute.

Finally, Zhang et al. [49], attempting to “bridge the gap” between the epidemiologic evidence of hematologic malignancies “due to [occupational] formaldehyde exposure” and “possible mechanistic routes” conjectured that inhaled formaldehyde could cause leukemia by “inducing DNA damage and chromosome aberrations in hematopoietic stem or early progenitor cells in the bone marrow or circulating in the blood”. Placing the debatable and speculative aspects of this proposition aside [65], we do not think it is plausibly applicable to the circulating formaldehyde originating from vaccine administration for all of the reasons stated thus far, as well as the significant differences in rate and duration of exposure to formaldehyde that are known to exist between the occupational and iatrogenic exposure scenarios.

4. Conclusion

Formaldehyde is an effective cross-linking agent that is used in certain vaccines to inactivate viruses and to detoxify bacterial toxins while not materially affecting antigenicity. As a result of the manufacturing process, some residual formaldehyde can remain behind in certain vaccines at low levels. Vaccine formaldehyde has not been associated with local or systemic adverse effects other than a single case of exacerbation of eczema reported over 25 years ago in an adult healthcare worker who received a formalin-containing hepatitis B vaccine. As part of a rigorous and ongoing process of evaluation of the safety of biological products throughout their lifecycle, we developed a physiologically-based pharmacokinetic model of vaccine formaldehyde disposition following intramuscular injection of a model 2-month-old infant. Our model indicated that following a single dose of 200 µg, formaldehyde is essentially completely removed from the site of injection within 30 min. Under the conservative assumption of metabolism at the site of injection only, peak concentrations of formaldehyde in blood/total body water were estimated to be 22 µg/L, which is equivalent to a body burden that is <1% of the endogenous level of formaldehyde. Predicted levels in the lymphatics were even lower. In the absence of any known adverse health effects from endogenously produced formaldehyde, which exists in blood and extravascular water at steady-state concentrations that are much lower than 100-fold higher, we consider vaccine-related, exogenous formaldehyde to be a miniscule part of the daily formaldehyde turnover by the body, and, therefore, do not find it plausible that vaccine-related formaldehyde represents an unsafe component of infant vaccines.

Acknowledgment

We thank Dr. Agnieszka Sulima for assistance with her translation of reference 58 from Polish.

References


