Adjuvanticity and Inflammatory Response Following Administration of Water-in-Oil Emulsions Prepared with Saturated Hydrocarbons in Chickens

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ABSTRACT. Water-in-oil emulsions containing bovine serum albumin (BSA) as a model antigen were prepared using aliphatic saturated hydrocarbons with carbon number from 12 to 18, and were tested in chickens. Straight-chain hydrocarbons induced higher antibody titers against BSA after administration than branched-chain hydrocarbons. \(n\)-C\(_{16}\)H\(_{34}\) and \(n\)-C\(_{18}\)H\(_{38}\) maintained high antibody titers even at 32 weeks after administration, compared with \(n\)-C\(_{12}\)H\(_{26}\), \(n\)-C\(_{14}\)H\(_{30}\) and \(n\)-C\(_{15}\)H\(_{32}\). \(n\)-C\(_{12}\)H\(_{26}\) and \(n\)-C\(_{14}\)H\(_{30}\) raised concentrations of sialic acid and creatine kinase in plasma, both of which are important markers of inflammatory responses, immediately after administration. \(n\)-C\(_{16}\)H\(_{34}\) and \(n\)-C\(_{18}\)H\(_{38}\) did not raise the values of these markers. These results indicated that \(n\)-C\(_{16}\)H\(_{34}\) and \(n\)-C\(_{18}\)H\(_{38}\) induced elevated and sustained immune responses without severe adverse reactions in chickens.

KEY WORDS: adjuvanticity, hydrocarbon, inflammation.


Oil adjuvanted vaccines, formulated as water-in-oil (W/O) emulsions, have been used to enhance immune responses in poultry [2, 3, 6]. However, W/O emulsion vaccines containing liquid paraffin often cause sterile granulomas at the site of injection in chickens [1, 9]. Stewart-Tull et al. reported that both immune response and side effects of W/O emulsions varied with the type of liquid paraffins used (e.g. Difco, Nacalai and Esso Bayol F) in guinea pigs [5]. They assumed that these variations were due to difference in hydrocarbon compositions between the different types of liquid paraffin. Hence, it is necessary to elucidate the action of various saturated hydrocarbons for selection of liquid paraffin. However, the relationship between immune or inflammatory responses and hydrocarbons in poultry has not been reported in detail.

We investigated adjuvanticity and inflammatory responses induced by W/O emulsions containing bovine serum albumin (BSA) (Seikagaku Co., Japan) prepared with various aliphatic saturated hydrocarbons, straight- and branched-chain hydrocarbons with carbon numbers from 12 to 18 (Wako Pure Chemical Industries, Ltd., Japan) (Table 1), after intramuscular injection in chickens.

W/O emulsions were prepared as follows: The aqueous phase (30 parts) composed of BSA and Dulbecco’s phosphate-buffered saline (-) at pH 7.4 (PBS) was slowly added to the oil phase (70 parts) composed of the hydrocarbons, sorbitan sesquioleate (Kao Chemicals, Japan) and polysorbate 80 (Kao Chemicals, Japan) in a 65:4.5:0.5 ratio. Then, the mixture was sonicated using an ultrasonic disrupter UR-200P model (Tomy Seiko Co., Japan) below 25°C for emulsification. The BSA content in the formulations was 0.2 mg/ml.

Specific-pathogen-free (SPF) Aburahi chickens, 4–5 weeks old, were obtained from the SPF flock at Aburahi Laboratories, Shionogi Co., Ltd. (Japan). Chickens were reared in isolation facilities maintained at a constant temperature. W/O emulsions (0.5 ml/chicken) were injected into the thigh muscles of chickens (5 birds/group). Blood samples were obtained for determination of the anti-BSA antibody titer and the concentrations of sialic acid and creatine kinase.

Anti-BSA antibody titer was detected by an enzyme-linked immunosorbent assay (ELISA). Ninety-six well immunoplates (Falcon 3072, Becton Dickinson Co., France) were coated overnight at 4°C with 100 µl/well of 2% BSA in carbonate-bicarbonate buffer at pH 9.6. The plates were washed with PBS containing 0.1% Tween 20 as a washing buffer. After adding blocking buffer consisting of PBS containing 3% skim milk, the plates were washed. The diluted test serum was added and followed by incubation for two hours at room temperature. The plates were washed, and a 1:2500 dilution of

<table>
<thead>
<tr>
<th>Hydrocarbon compound</th>
<th>Molecular weight</th>
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<tbody>
<tr>
<td>Dodecane ((n)-C(<em>{12})H(</em>{26}))</td>
<td>170.3</td>
</tr>
<tr>
<td>Tetradecane ((n)-C(<em>{14})H(</em>{30}))</td>
<td>198.4</td>
</tr>
<tr>
<td>Pentadecane ((n)-C(<em>{15})H(</em>{32}))</td>
<td>212.4</td>
</tr>
<tr>
<td>Hexadecane ((n)-C(<em>{16})H(</em>{34}))</td>
<td>226.5</td>
</tr>
<tr>
<td>Octadecane ((n)-C(<em>{18})H(</em>{38}))</td>
<td>254.5</td>
</tr>
<tr>
<td>2,2,4,6,6-Pentamethylheptane ((n)-C(<em>{12})H(</em>{26}))</td>
<td>170.3</td>
</tr>
<tr>
<td>2,2,4,4,6,8,8-Heptamethylnonane ((n)-C(<em>{18})H(</em>{38}))</td>
<td>226.5</td>
</tr>
</tbody>
</table>
peroxidase-conjugated sheep anti-chicken IgG was added to each well and incubated for one hour at room temperature. After washing, o-phenylene diamine dihydrochloride [0.02% in citrate-phosphate buffer, 0.004 vol% H2O2] was used as a substrate. After five minutes, the reaction was stopped by the addition of 2 N sulfuric acid. Absorbency in each well was determined with a microplate autoreader (Multiskan® Bichromatic; Labosystems Japan Co., Japan) at 492 nm.

Sialic acid and creatine kinase concentrations in plasma, both of which are important markers of inflammatory responses, were measured by enzymatic assay used a sialic acid test pack (Kyokuto Co., Japan) [7] and according to the procedure of Szasz et al. [8] using an ABBOTT VISION creatine kinase test pack (Dainabot Co., Japan), respectively.

Statistical significance was assessed by Student’s paired t-test. A p value of <0.05 was considered significant.

Among W/O emulsions prepared using hydrocarbons with the same molecular weight, straight-chain hydrocarbons induced significantly (p<0.05) higher antibody titers against BSA at 8 and 12 weeks after administration than branched-chain hydrocarbons (Table 2). n-C12H26, n-C14H30, n-C15H32, n-C16H34 and n-C18H38 induced significantly (p<0.05) high antibody titers at 4 weeks after administration compared with n-C12H26 (Fig. 1). n-C16H34 and n-C18H38 maintained significantly (p<0.05) high values even at 32 weeks after administration compared with the other hydrocarbons. On the other hand, n-C14H30 and n-C15H32 did not show a high and sustained plateau level during the 32-week experimental period. These results were similar to those reported previously in guinea pigs [4], although this previous report did not describe the maintenance of immune responses.
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responses. Our results indicated that \( n\text{-C}_{16}H_{34} \) and \( n\text{-C}_{18}H_{38} \) induced elevated and sustained immune responses against BSA in chickens.

\( n\text{-C}_{16}H_{34} \) and \( n\text{-C}_{18}H_{38} \) markedly increased both concentrations of sialic acid and creatine kinase within one or two weeks after administration, but \( n\text{-C}_{12}H_{26} \) and \( n\text{-C}_{14}H_{30} \) did not (Figs. 2, 3). Hence, \( n\text{-C}_{16}H_{34} \) and \( n\text{-C}_{18}H_{38} \) evoked strong inflammatory reactions immediately after administration. Inflammatory reactions of short-chain hydrocarbons with backbones of less than 14 carbons are thought to result in severe tissue destruction due to their lipid solvent action.

Consequently, \( n\text{-C}_{16}H_{34} \) and \( n\text{-C}_{18}H_{38} \) in liquid paraffin were found to induce elevated and sustained immune responses without inducing severe adverse reactions in chickens. Our results indicated that it is necessary to select liquid paraffin chiefly consisting of \( n\text{-C}_{16}H_{34} \) and/or \( n\text{-C}_{18}H_{38} \) for use in preparation of W/O emulsions.

REFERENCES