Effect of Age and Degree of Immune Activation on Cytochrome P450 3A4 Activity After Influenza Immunization

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Study Objective. To measure age- or sex-related changes in cytochrome P450 (CYP) activity secondary to influenza vaccination.

Design. Open-label, single-dose study.

Setting. General clinical research center at a university hospital.

Subjects. Fifteen healthy volunteers aged 22–51 years.

Intervention. Each subject was given an erythromycin breath test (ERMBT) to measure CYP3A4 activity before influenza immunization and again on day 7 after immunization. Blood was drawn before immunization and on day 28 after immunization to measure influenza antibody concentrations.

Measurements and Main Results. Age of subject and change in ERMBT results after influenza immunization were correlated (correlation coefficient -0.624, p<0.015). However, no correlations could be made between antibody concentrations after influenza immunization or change in antibody concentrations from baseline and age.

Conclusion. Decreases in CYP3A4 activity after influenza immunization are associated with increasing age. The decreases in CYP3A4 activity, however, are not associated with influenza antibody concentrations. This study bears repeating in an older cohort since the study sample did not include elderly individuals.

(Pharmacotherapy 2002;22(10):1235–1238)

Reports of changes in cytochrome P450 (CYP) activity after influenza vaccination are conflicting. In two studies, clearance of anticonvulsants, phenytoin, carbamazepine, and phenobarbital decreased within 1–2 weeks after influenza vaccination.1, 2 Another study reported variable changes in phenytoin concentrations, with significant increases in concentrations in a few individuals.3 A case report describes an example of an adverse reaction induced by influenza immunization when rhabdomyolysis developed in a patient who was taking cerivastatin.4 Most statins are CYP3A substrates, and routine therapeutic drug monitoring is not done. In addition, clearance of theophylline, a CYP1A2 substrate, decreased or remained unchanged after influenza immunization in several studies.5–8 These inconsistent findings may be due to the heterogeneity in the populations studied.

In general, findings from studies of healthy individuals demonstrate no changes in drug metabolism after influenza vaccination.6, 8–11 However, studies of infirm individuals—those for whom annual influenza immunization is highly
recommended—show decreased clearance of drugs.\textsuperscript{1, 2, 5} Two studies of elderly individuals showed interesting trends toward decreased phenytoin clearance after influenza immunization.\textsuperscript{3, 7} There may be a group of individuals who are particularly susceptible to changes in drug-metabolizing capacity after influenza immunization.

No decrease in CYP3A4 activity with increasing age has been documented.\textsuperscript{12, 13} However, whether increasing age makes individuals more or less susceptible to enzyme induction or inhibition is unknown. Influenza vaccine is indicated for all individuals aged 50 years or older.\textsuperscript{14} Many patients in this age group take many drugs, which emphasizes the importance of characterizing mechanisms of potential drug interactions.

The CYP3A4 enzyme is responsible for the metabolism of a significant fraction of all drugs in clinical administration and development.\textsuperscript{15, 16} Changes in CYP3A4 activity have potential clinical importance. We administered the erythromycin breath test (ERMBT) as a probe of CYP3A4 to measure changes in activity after influenza immunization. This test estimates in vivo catalytic activity of hepatic CYP3A4 by measuring the radiolabeled carbon dioxide that is exhaled after a 3-µCi (< 1 mg) intravenous dose of [\textsuperscript{14}C-N-methyl] erythromycin.\textsuperscript{15, 17} The ERMBT is useful for monitoring CYP3A4 activity before and after an intervention.\textsuperscript{18} We found a mean change of \(-4\%\) in ERMBT results after influenza immunization, which was not statistically significantly different from the baseline measurement.\textsuperscript{9} However, large changes in CYP3A4 activity occurred in some individuals. Our hypothesis was that these changes may be correlated with age or immune activation.

Methods

We enrolled healthy adults before the influenza season in Wisconsin (October–mid-November 1998). Individuals aged 18–60 years who had no acute or chronic illness and were not taking any drugs were eligible. Each subject gave written informed consent to participate in this open-label, single-dose study, which was approved by the University of Wisconsin–Madison Human Subjects Committee. All study visits occurred at the University of Wisconsin General Clinical Research Center. Subjects were instructed not to change their diets, and to avoid grapefruit juice.

Study subjects were given a baseline ERMBT, and an influenza antibody concentration was measured before they received the 1998–1999 influenza vaccine 0.5 ml intramuscularly (Connaught Laboratories, Inc., Swiftwater, PA). The methods for performing the ERMBT and measuring influenza antibody concentrations have been described elsewhere.\textsuperscript{9} Subjects returned to the clinic 7 days after vaccination for a second ERMBT and on day 28 for another measurement of influenza antibody concentrations. Blood samples were collected and processed, and plasma was separated and frozen for later use.

The study was designed with 80% power and an \(\alpha\) of 0.05 to detect a 20% change in ERMBT results after influenza immunization. Spearman rank order was used to determine correlations between changes in ERMBT results and age of subject.

Results

Fifteen healthy subjects—eight men and seven women aged 22–51 years (mean 31.9 yrs, standard error 2.6)—completed the study with no reports of adverse effects.

We found a significant inverse correlation between age of subject and change in ERMBT result (correlation coefficient \(-0.624, p<0.015\); Figure 1). Older individuals had significantly higher degrees of CYP3A4 inhibition after influenza immunization.

Each subject was given two ERMBTs, one before and one after immunization. The test quantifies CYP3A4 activity by measuring the production of \textsuperscript{14}C-labeled carbon dioxide in the

![Figure 1](image_url)

**Figure 1.** Age of subjects and change in cytochrome P450 (CYP) 3A4 activity after influenza immunization. Change in erythromycin breath test (ERMBT) results correlated with age (correlation coefficient \(-0.624, p<0.015\), Spearman rank order). Changes in CYP3A4 activity showed that it decreased with increasing age after influenza immunization.
exhaled breath. Since the increasing susceptibility to enzyme inhibition may be related to a baseline change in CYP3A4 activity with age, we also examined a possible correlation between age and CYP3A4 activity. However, consistent with findings from other work, no age-related decline was observed in CYP3A4 activity.12 Stated another way, we saw no correlation in baseline or postimmunization ERMBT results with age (p=0.67 for both comparisons). In addition, we found no associations between sex and ERMBT results.

As a measure of immune activation, we measured antibody concentrations by hemagglutination inhibition assay. We also found no correlation between postinfluenza immunization titer or change in titer from baseline and age for any of the various strains of influenza viruses (p>0.3 for all comparisons; Figure 2). Neither antibody concentrations after immunization nor change in antibody concentrations were associated with a change in ERMBT results.9

**Discussion**

Our study shows that decreases in CYP3A4 activity secondary to influenza immunization are associated with increasing age. This association warrants additional study in an older population, for whom annual influenza vaccination is recommended. The timing and duration of the changes may be important as well.

Immune activation may be responsible for the change in drug metabolism after vaccination. The hypothesis that cytokine production affects the expression or activity of CYP enzymes has been made several times. Direct treatment of human and animal hepatocytes in primary culture with cytokines alters both the expression and the activity of CYP enzymes.19–23 In addition, the change in carbamazepine (a CYP3A4 substrate) pharmacokinetics after neurosurgery is associated with serum interleukin-6 levels.24

The lack of association of influenza antibody response with age in our study is not surprising. Studies finding decreased antibody response to influenza vaccine with age have involved subjects older than 65 years,25–27 whereas our oldest subject was 51 years. Neither the age range of our subjects nor the sample may be large enough to demonstrate a decline in influenza antibody concentrations.

**Conclusion**

We noted an interesting inverse correlation between increased age of subjects and decreased CYP3A4 activity after influenza immunization. As many older individuals take many drugs, this finding becomes particularly important—not just for examining a change in metabolic capacity induced by influenza vaccine, but for evaluating the susceptibility of elderly individuals to drug interactions. Because annual influenza immunization for all individuals aged 50 years or older is recommended, this finding bears confirmation in a study of this age group.

**References**