Efficacité de la réponse immune post vaccination lors d'un traitement par statine

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EFFICACITE DE LA REPONSE IMMUNE POST VACCINATION LORS D'UN TRAITEMENT PAR STATINE

Thèse
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TABLE DES MATIERES

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LE TRAITEMENT ANTI-INFLAMMATOIRE DE L'ATHEROSCLEROSE

Introduction

La vision traditionnelle de l'athérosclérose comme une simple maladie de stockage du cholestérol s'écroule à l'aune des preuves extensives que l'inflammation joue un rôle central dans toutes les étapes de cette pathologie, de la lésion initiale aux complications thrombotiques finales et dévastatrices [1] [2]. L'application clinique du concept de l'inflammation comme facteur de risque cardiovasculaire a engendré l'identification de biomarqueurs de l'inflammation tels que la CRP (protéine C-réactive), le CD40ligand soluble, l'adiponectine, l'IL (interleukine)-18 et la MMP (matrix metalloproteinase)-9 comme prédicteurs de risque cardiovasculaire [3].

L'athérosclérose peut se manifester cliniquement par des symptômes chroniques tels que l'angor stable ou la claudication intermittente, de manière aiguë par un infarctus du myocarde ou un accident cérébro-vasculaire, voire rester cliniquement silencieuse. Malgré une large panoplie de traitements pharmacologiques et interventionnels pour combattre ce fléau, l'athérosclérose reste la cause première de mortalité et de morbidité dans le monde développé.

Interventions Pharmacologiques

Les facteurs de risque classiques de l'athérosclérose doivent en premier lieu et avant tout être combattu par une modification des habitudes de vie, tels que l'alimentation, l'activité physique et l'arrêt du tabagisme. En effet, malgré une amélioration des symptômes de l'angine de poitrine, un simple traitement des vaisseaux sanguins sténosés par des procédures interventionelles tels que l'angioplastie ou le pontage coronarien ne prolongent guère la durée de vie des patients.

Le traitement pharmacologique des facteurs de risque par des médicaments anti-hypertenseurs, hypolipémiants, anti-diabétiques et anti-plaquettaires constituent l'étape suivante de la prévention primaire et secondaire de l'athérosclérose, habituellement initiés avec des modifications de mode de vie. En particulier, certains médicaments peuvent modifier et atténuer certains processus inflammatoires qui œuvrent dans l'athérosclérose [4]. La revue suivante souligne les effets anti-inflammatoires — souvent imprévues — de médicaments anti-athéroscléreux.

Statines (inhibiteurs de la 3-hydroxy-3-methylglutaryle coenzyme A réductase)

Cette classe de médicaments hypolipémiants abaisse efficacement les taux de cholésterol LDL (low-density lipoprotein) et réduit significativement les complications cardiovasculaires [5] [6]. Il est important de noter que les statines n'ont qu'un effet modeste sur le niveau de sténose artérielle [7], ce qui souligne que le degré d'activation inflammatoire de la plaque athéroscléreuse, et non son volume, détermine sa propension à engendrer un syndrome coronarien aigu. En effet, les taux circulants de plusieurs marqueurs inflammatoires qui sont associés avec un risque cardiovasculaire accru, en particulier la CRP, sont modifiés suite à un traitement par statines [3]. En outre, la CRP constitue un facteur de risque indépendant et améliore la capacité prédictive des marqueurs traditionnels de risque cardiovasculaire chez la femme [8] et l'homme [9].

Des preuves supplémentaires venant de la recherche clinique soutiennent le rôle anti-inflammatoire et immunomodulateur des statines. La diminution de risque associé à un traitement par statine dépasse celui attendu par une simple baisse des taux de cholestérol LDL. L'étude CARE (Cholesterol And Recurrent Events) a été la première à démontrer que la thérapie par statine diminue non seulement les taux de cholestérol LDL, mais également la concentration plasmatique de CRP [10]. Des analyses rétrospectives ont soutenus l'utilisation de ce marqueur inflammatoire comme cible des statines chez les patients normocholestérolémiques, tant pour la prévention cardiovasculaire primaire [11] que secondaire [12]. De surcroît, l'étude JUPITER (Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin) a récemment démontré, de manière prospective, que les patients ayants des taux de cholestérol LDL considérés comme optimales, mais avec des concentrations de CRP élevées, bénéficient de manière significative d'un traitement par statine pour la prévention cardiovasculaire primaire [13], un résultat ayants de vastes conséquences pour la prise en charge clinique des patients.

L'effet anti-inflammatoire des statines résulte probablement de leur capacité à inhiber la formation de l'acide mévalonique [14]. Les produits en aval de cette molécule comprennent non seulement le produit final qu'est le cholestérol, mais également plusieurs isoprénoides qui modifient par prénylation de manière covalente certaines molécules cruciales de signalisation intracellulaire. Le traitement par statines inhibe également l'adhésion des leucocytes aux plaques [15], l'accumulation de macrophages à

l'intérieur des lésions [16], ainsi que la production de protéases [17 18 19], du facteur tissulaire [20 21] ainsi que d'autres médiateurs pro-inflammatoires. En agissant sur le transactivateur du MHC (major histocompatibility) de classe II (CIITA), les statines inhibent également la présentation d'antigènes et l'activation des cellules T [22].

Ces résultats soutiennent le concept qu'outre leur effet favorable sur le profil lipidique, les statines exercent également une panoplie d'actions anti-inflammatoires et immuno-modulatrices [23].

Les agonistes des PPAR (Peroxisome Proliferator-Activated Receptor)

Les PPARs appartiennent à la famille des récepteurs nucléaires, et comprennent 3 isoformes ($\alpha, \gamma,$ et δ). Les activateurs du PPAR-α (les fibrates tels que le gemfibrozile ou le fenofibrate) agissent en diminuant les taux de triglycérides et en augmentant ceux du cholestérol HDL (high density lipoprotein), alors que les activateurs du PPAR-γ (les thiazolidinediones tels que la pioglitazone ou la rosiglitazone) sont des agents augmentant la réponse à l'insuline. L'identité des ligands endogènes aux PPARs n'est toujours pas élucidée, mais des travaux récents suggèrent un rôle des lipases tels que la lipoprotéine lipase et la lipase endothéliale ainsi que des substrats appartenant à la famille des lipoprotéines tels que les VLDL (very low density lipoprotein) [24] et HDL [25] pour générer ces ligands.

Plusieurs résultats suggèrent que l'activation des PPARs inhibe l'inflammation et donc l'athérosclérose [26]. La PPAR-α ainsi que la PPAR-γ réduisent l'activation des cellules T [27], par exemple en diminuant la production de l'IFN (interféron)-γ. Les agonistes de la PPAR-α bloquent l'expression endothéliale de VCAM (vascular cell adhesion molecule)-1 [28] et inhibent l'activation inflammatoire des cellules musculaires lisses vasculaires [29], alors que les agonistes de la PPAR-γ bloquent l'expression endothéliale de chémokines [30] et diminuent la production de MMPs (matrix metalloprotease) par les macrophages [31].

Chez l'humain, la variation des biomarqueurs en réponse aux agonistes des PPARs semble confirmer ces possibles effets anti-athéroscléreux, bien que les résultats de certaines études cliniques récentes n'aient pas été conclusives [26]. De futures études

cliniques à grande échelle devraient nous aider à définir le rôle de la thérapie avec des agonistes des PPARs chez les patients ayant un risque élevé de développer un accident cardiovasculaire.

La régulation des mastocytes

Des expériences récentes ont élucidées le rôle délétère de l'activation mastocytaire dans l'athérosclérose expérimentale. Les athéromes de souris mutantes déficientes en mastocytes et prônes à développer des lésions athéroscléreuses exhibent des lésions plus limitées, avec une moindre déposition de lipides, cellules T, macrophages, mais avec des capes fibreuses enrichies en collagène et donc plus robustes [32]. De manière importante, le traitement des souris athéroscléreuses avec le stabilisateur mastocytaire disodium cromoglycate engendre des résultats similaires [33]. Cet agent bloque le largage par les mastocytes de leurs granules, qui contiennent des agents tels l'IL (interleukine)-6 et l' IFN-γ qui activent d'autres cellules inflammatoires présentes dans l'athérome. De surcroît, le traitement avec le disodium cromoglycate diminue les taux de cathepsines et MMPs au sein des plaques, engendrant un phénotype ayant les caractéristiques de lésions humaines considérées comme stables.

Etant donné l'usage routinier des stabilisateurs mastocytaires en clinique, par exemple dans le cadre du traitement de l'asthme, ces résultats expérimentaux devraient stimuler une évaluation clinique chez l'humain.

Les récepteurs aux cannabinoïdes

Des 2 types de récepteurs aux cannabinoïdes connus, le CB2 est exprimé de manière préférentielle sur les cellules immunes [34]. En recherche fondamentale, l'activation du CB2 améliore l'inflammation chronique de maladies telles que l'arthrite rhumatoïde. De récents résultats suggèrent que les cannabinoïdes ont également un effet bénéfique dans l'athérosclérose. Dans les lésions athéroscléreuses, le Δ9-tetrahydrocannabinol (THC) bloque la sécrétion d'IFN-γ par les cellules T et réduit l'infiltration des macrophages en inhibant l'expression du récepteur des chémokines CCR2 [35]. De manière intéressante, de récents résultats suggèrent que les cannabinoïdes sont des ligands du PPAR-γ [36]. Des résultats expérimentaux plus amples ainsi que de futures

validations cliniques détermineront si les cannabinoïdes feront leur entrée dans la catégorie des thérapies anti-athéroscléreuses.

Conclusion

La réponse immune est un élément central de l'athérosclérose, de l'initiation en passant par la progression jusqu'aux complications thrombotiques [1] [2]. En effet, notre compréhension accrue de la biologie de ce fléau a beaucoup évolué aux cours des dernières décennies, et nous a progressivement éloigné de la perspective ultérieure qui considérait l'athérosclérose comme une maladie consistant exclusivement une pathologie d'accumulation non contrôlée de cholestérol. Nous comprenons maintenant que c'est l'état d'activation inflammatoire de la plaque athéroscléreuse, qui aboutit à une cape fibreuse fine prône à la rupture, et non le degré de sténose artérielle, qui influence les manifestations cliniques de cette maladie [37] [38]. Cette compréhension accrue de la biologie de la plaque nous permet de disséquer les manières diverses par lesquelles l'athérosclérose peut se manifester cliniquement, et pourquoi cette maladie peut rester silencieuse pendant de longues périodes et être interrompues par des complications aiguës.

Ces nouvelles connaissances du rôle de l'inflammation dans l'athérosclérose nous permettent progressivement de traduire ces compréhensions du domaine expérimental à la pratique clinique, par exemple en nous aidant à identifier des individus à haut risque de complications cardiovasculaires. Dans ce contexte, des biomarqueurs inflammatoires tels la CRP méritent d'être considérés de manière rigoureuse dans des stratégies de stratification de risque. De plus, ces avancées scientifiques nous offrent un cadre pour mieux comprendre les mécanismes par lesquels les modifications de style de vie et certaines thérapies médicales tels les statines diminuent l'inflammation, stabilisent les plaques, et ce faisant réduisent les épisodes cliniques adverses.

Autorisation de la commision d'éthique des HUG

L'étude clinique suivante a été menée aux Hôpitaux Universitaires de Genève au sein de la Division de Cardiologie du Département de Médecine Interne en collaboration avec le Centre de Vaccinologie et d'Immunologie Néonatale du Département de Pédiatrie.

La Commission d'Ethique du Département de Médecine Interne des HUG, présidée par Monsieur le Professeur René Rizzoli, ayant donné son accord pour la réalisation de cette étude (Etude Statines — Vaccination CE: 04-029).

Résumé

De nombreuses études expérimentales et cliniques ont démontré qu'en plus de leurs effets hypocholestérolémiques, les statines exercent également des actions anti-inflammatoires et immunomodulatrices, soulevant l'hypothèse que les statines puissent moduler, positivement ou négativement, la production d'anticorps lors d'une vaccination.

150 volontaires sains dépourvus d'immunité contre l'hépatite A furent recrutés et leur réponse à la vaccination contre l'hépatite A fut évaluée lors d'un traitement avec l'atorvastatine (40mg par jour pendant 28 jours) dans une étude clinique randomisée en double-aveugle avec groupe de contrôle placébo. L'atorvastatine n'a pas influencé de manière significative la séroconversion après vaccination contre l'hépatite A comparée au placébo. Des proportions similaires de participants ont atteint la séroprotection : 92,9% du groupe contrôle comparé à 97,0% du groupe atorvastatine (P=0,41).

En conclusion, la thérapie par statine ne compromet pas le processus d'immunisation et la production d'anticorps qui en résulte, et devrait en conséquence être continuée lors d'une vaccination.

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Atorvastatin Treatment and Vaccination Efficacy

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ABSTRACT

Background

Experimental and clinical data have demonstrated that in addition to their hypocholesterolemic effects, statins also exhibit anti-inflammatory and immunomodulatory properties. While the adaptive immune response requires T helper lymphocyte activation and a certain pattern of inflammatory cytokine expression, CD4+ Th2 cells play a crucial role in supporting the differentiation of B cells to antibody-secreting plasma cells, raising the hypothesis that statins may modulate, either negatively or positively, antibody responses to vaccine antigens.

Methods

We enrolled 150 healthy volunteers lacking immunity to hepatitis A and evaluated their antibody response to hepatitis A vaccination while on atorvastatin treatment in a double-blind, randomized, placebo-controlled trial. Subjects were immunized against hepatitis A and subsequently received atorvastatin (40 mg per day) or placebo for a period of 28 days after immunization.

Results

Treatment with atorvastatin 40 mg daily did not significantly influence blood seroconversion levels after hepatitis A immunization compared to placebo. Similar proportions of participants achieved seroprotection: 52 of 56 controls (92.9%) versus 65 of 67 in the atorvastatin group (97.0%, p=0.41). Total blood cholesterol, LDL-cholesterol, triglycerides and C-reactive protein values did not change in the placebo group, but were significantly reduced in all participants who received atorvastatin.

Conclusion

Despite growing evidence that statin therapy can induce anti-inflammatory and immunomodulatory effects, and might thus impair effective vaccination, treatment with atorvastatin 40 mg per day did not influence the antibody response after vaccination. Thus, we conclude that statin therapy does not compromise an immunization process and should be continued throughout vaccination.

INTRODUCTION

In the last decades, substantial progress has been made in understanding the relationships between lipid disorders, atherosclerosis and the prevention of cardiac ischemic disease [39]. 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, have been described as the principal and most effective class of drugs to reduce serum cholesterol levels as well as cardiovascular events in patients with or without coronary artery disease [40, 41].

Interestingly, in addition to their cholesterol lowering effect, statins have also been shown to exhibit anti-inflammatory and immunomodulatory properties [23]. Several statins were shown to directly inhibit the induction of major histocompatibility complex class II (MHC-II) expression, leading to a repression of MHC-II mediated T-cell activation, an essential step in the induction of an immune response such as vaccination [22]. A decrease in the cellular expression levels of CD40, CD40L as well soluble CD40L has also been observed [42]. Statins have been shown to interfere with the expression of pro-inflammatory cytokines as well as C-Reactive Protein (CRP) [43]. In addition, in an experimental animal model of multiple sclerosis, atorvastatin promoted the differentiation of Th0 cells into Th2 cells [44]. It was proposed that these initially unexpected anti-inflammatory and immunomodulatory effects of statins, blocking aberrant activation of T lymphocytes and favoring a Th2 phenotype, should be beneficial in auto-immune diseases and in organ rejection following transplantation. Indeed simvastatin was reported to markedly inhibit collagen-induced arthritis in mice [45]. At the clinical level, two trials have suggested that statins exert beneficial effects in patients after cardiac transplantation [46, 47]. Recently, clinical studies reported that atorvastatin exerts a beneficial effect in rheumatoid arthritis [48], ankylosing spondylitis [49], and that treatment with simvastatin over a 6 month period markedly inhibits the inflammatory components of multiple sclerosis [50]. All these results may provide a rationale for the use of statins as novel immunomodulators and anti-inflammatory agents in immuno-inflammatory diseases [23].

However, the influence of statin treatment on B lymphocyte function was never assessed. T helper lymphocyte responses play a crucial role in supporting the differentiation of B lymphocytes towards antibody-secreting plasma cells, raising the important hypothesis that statin treatment may indeed modulate antibody responses to vaccine antigens. Whether such a modulation would have a beneficial influence (as

Th2 responses tend to provide better support to B lymphocyte differentiation) or whether inhibition of T helper lymphocytes, through statin-induced decrease of MHC Class II expression and broad anti-inflammatory effects, may possibly exert a deleterious influence on responses to vaccine antigens is difficult to predict on the basis of existing data.

Given the rapidly increasing use of statins by a population of patients immunized yearly against the influenza virus and other pathogens, we aimed to investigate whether statin treatment may influence the immune response after vaccination through a randomized, double-blinded, placebo-controlled clinical trial. The hepatitis A vaccine was selected for its strong immunogenicity, eliciting seroconversion after a single vaccine dose in most seronegative healthy subjects, and for its relevance as a vaccine increasingly recommended to travelers, a growing fraction of which benefit from statin therapy.

METHODS

Participants

Between November 2004 and June 2005, 150 healthy volunteers underwent randomization at the Division of Cardiology, Department of Medicine, Geneva University Hospital (HUG). Men and women who were ≥18 years old were eligible for inclusion if they had neither morbidities nor immunity to hepatitis A. Exclusion criteria were anti-hepatitis A antibodies >10 IU/L, hypercholesterolemia, hepatitis, myositis, chronic alcohol abuse, pregnant or breast-feeding women and volunteers on drug therapy aside oral contraceptives. The protocol was approved by the Ethics Committee of the HUG, and written informed consent was obtained from all participants. Of the 150 healthy volunteers enrolled, 123 completed the study protocol, 67 and 56 participants in the atorvastatin and placebo group, respectively (Figure 1). Characteristics of the participants are shown in Table I. The two groups of participants were similar, with an average age of 24 years, a mean body mass index of 21.7 kg/m^2 and 55 percent were women. Factors known to affect anti-HAV vaccine response essentially include gender and body mass index [51]. These parameters were similar between the atorvastatin and placebo groups (Table I).

Study protocol

150 boxes containing 28 capsules each were randomized by use of a predefined algorithm to either atorvastatin 40 mg or placebo by the Division of Pharmacy at the HUG and were randomly given to the investigators. Thus, during the study period, neither the investigators nor the volunteers knew which treatment was assigned.

On day 1, participants underwent a blood puncture to confirm the absence of hepatitis A immunity (anti-hepatitis A antibodies <10 IU/L). Eligible participants were immunized intramuscularly with 1 dose of hepatitis A vaccine (Havrix-1440®; Glaxo Smith Kline) and received a box containing 28 capsules each of either atorvastatin 40 mg or placebo with the instruction to take one capsule every evening as of day 1 of enrollment. On day 29, after 28 days of treatment, participants underwent a second blood puncture to determine hepatitis A immunity by measuring anti-hepatitis A antibody titers. Blood samples were analyzed at the laboratory of the Center for Vaccinology and Neonatal Immunology, Faculty of Medicine, University of Geneva. Antibodies to hepatitis A virus antigen were analyzed using the

EnygnostR Anti-HAV test kit from Dade Behring Marburg GmbH, Marburg, Germany. The serum samples were stored frozen until use and analyzed after appropriate dilution using the kit as a manual, quantitative test according to the manufacturer's instructions. Antibody concentrations were calculated with the Softmax® PRO software (Molecular Devices) by comparison with the standard curve of the test kit by four-parameter fitting. Values were given as IU/L. Vaccine responses were defined individually, in a blinded manner.

Outcome variables

The main outcome variable was the achievement of antibody levels >20 IU/L against the hepatitis A virus one month after vaccination. A secondary outcome variable was the mean log-transformed antibody titer.

To document effects of atorvastatin, total blood cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides were measured at the Clinical Chemistry Core Facility of the HUG, and high-sensitive CRP (hsCRP) values were measured using ELISA at the Foundation for Medical Research, University of Geneva. All these parameters were measured at randomization and at the end of the study in a blinded manner.

Statistical analysis

We compared the results of treatment with atorvastatin with those of placebo on the antibody response to hepatitis A vaccination. Assuming a type 1 error of 0.05, the trial had a power of 90% to identify a decrease of more than 10% in the mean antibody concentration of individuals in the atorvastatin group. This was based on prior data with an estimate of mean antibody concentration after seroconversion against the hepatitis A virus of 250 IU/L with a standard deviation of 40 IU/L. We would consider a negative difference in concentration of >10 % in the statin group as significant, i.e. >25 IU/L, or about 0.6 standard deviations [52]. Immunity to hepatitis A immunization was assessed by comparing: the % of sero-responders (defined as % of subjects reaching the assay cut-off), the % of sero-protected individuals (defined as % of subjects reaching antibody titers >20 IU/mL) and antibody titers elicited 28 days after immunization. Proportions were compared by means of the Fisher chi-square test. Untransformed antibody titers were compared using the Mann-Whitney test. Antibody titers were also log transformed to obtain roughly normally distributed

measures, and these geometric mean titers were compared using a t-test. Analyses were run on SPSS 11.5 for Windows. P values <0.05 were considered as statistically significant. The investigators designed the trial and had free and complete access to the data.

RESULTS

Two participants failed to respond to the hepatitis A vaccine, as reflected by their antibody levels inferior to 10 IU/L. Both were in the placebo group (failure of response to the hepatitis A vaccine = 2 of 56 in the placebo group versus 0 of 67 in the atorvastatin group, p=0.24) (Figure 2). This is consistent with a predicted 98-99% seroconversion rate after a single Havrix-1440® vaccine dose [53]. There was no increase or decrease in the primary end point, as reflected by the similar distribution patterns of serum antibody levels post vaccination (Figure 2). Similar proportions of participants achieved antibody levels exceeding 20 IU/L and were thus considered to be seroprotected: 52 of 56 controls (92.9%) versus 65 of 67 in the atorvastatin group (97.0%, p=0.41). Mean hepatitis A antibody titers at 29 days were 548.3 ± 607.7 IU/L in the atorvastatin group and 476.9 ± 438.3 IU/L in the placebo group, without statistical difference between the two groups (p=0.87). Investigating the different quartiles of hepatitis A antibody titer, no difference between atorvastatin and placebo treated participants was found (Figure 3). Differences in hepatitis A antibody responses between the 2 groups were also not found based on gender or smoking (data not shown).

Lipid values did not change in the placebo group, whereas all participants in the atorvastatin group had a significant reduction in total cholesterol (4.6 ± 0.7 to 3.0 ± 0.5 mmol/L), LDL-cholesterol (2.8 ± 0.5 to 1.4 ± 0.2 mmol/L) and triglycerides (1.0 ± 0.7 to 0.8 ± 0.4 mmol/L) (Table II), thus confirming compliance to treatment. When comparing the atorvastatin and placebo groups, total cholesterol and LDL-cholesterol were 1.8 ± 1.4 mmol/L and 1.6 ± 1.7 mmol/L lower at the end of the study (37.5% and 53.3% relative reduction, respectively) in the atorvastatin group, which was highly significant ($p<0.005$). Compared with placebo, atorvastatin also reduced triglycerides by 0.4 ± 0.1 mmol/L, a relative decrease of 33.3 % ($p<0.05$). Changes in HDL-cholesterol concentrations were minimal in the two groups and did not reach statistical significance. As for levels of hsCRP, there was a reduction in all participants in the atorvastatin group (1.69 ± 1.89 to 1.24 ± 1.28 mg/dL) ($p<0.02$) and a small non-significant increase in the placebo group (1.46 ± 1.95 to 1.61 ± 1.39 mg/dL), possibly due to the immune response to the hepatitis A vaccine. At the end of the 28 day period, the difference in hsCRP between the atorvastatin and placebo groups was statistically significant ($p<0.01$) (Table II).

DISCUSSION

3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or statins, have been described as the principal and most effective class of drugs to reduce serum cholesterol levels and have been shown to significantly reduce cardiovascular events and mortality [54, 55]. Recently, several trials emphasized the benefit of intensive statin therapy on reversing atherosclerosis [56] as well as providing greater protection against major cardiovascular events [57, 58].

In addition to their beneficial effects on the serum cholesterol profile, statins have been shown to have a multitude of other roles regrouped under the denomination of pleiotropic effects [14]. These inhibit several key components of atherothrombosis [59]. Consistent with these beneficial non-hypocholesterolemic effects, the secondary prevention of myocardial infarction with statin therapy showed that patients reaching low CRP levels have better clinical outcomes than those with higher CRP levels, regardless of the resultant level of low-density lipoprotein cholesterol [12]. Several trials have also investigated effects of statin therapy outside the vascular system, proving to be beneficial in patients after cardiac transplantation [46, 47] as well as improving clinical parameters in diseases such as rheumatoid arthritis [48], multiple sclerosis [50] and ankylosing spondylitis [49].

Thus, an ever increasing number of patients are on statin therapy, while in parallel, campaigns based on public health are becoming more insistent as regards immunization of the public in general and of the elderly in particular. Of note are the beneficial effects of influenza virus vaccination on the cardiovascular system, markedly decreasing death and myocardial infarction [60] as well as stroke [61].

The rationale for the present study was the concomitant action of statins as anti-inflammatory and immunomodulatory agents [42]. Statins have indeed been shown to exhibit numerous anti-inflammatory effects, primarily an inhibition of expression of MHC Class II molecules, resulting in impaired activation of T helper lymphocytes [22, 62], and inhibition of the expression of certain inflammatory cytokines [42, 43]. These effects could potentially interfere with the immunization process and thus prove to be deleterious for effective vaccination. However, statins have also been shown to behave as immunomodulatory agents, by favoring a Th2-type adaptive immune response [44, 63], which could prove to be beneficial to the immune response post vaccination.

To address the potential positive or negative effects of concomitant statin therapy on seroconversion rates post vaccination, we exclusively enrolled young healthy participants in our study so as not to take into account the presence of confounding factors such as age-related decline in the immune response, concomitant medications with secondary interactions as well as co-morbidities very often present in the elderly. The choice of atorvastatin was based on previous reports showing its having the most potent immunomodulatory effects within members of the statin family [22]. It was thought that a dose of 40 mg per day would prove sufficient to show an effect, if any, of atorvastatin on the immunization process since a 10 mg dose of the same drug was shown to significantly reduce cardiovascular events [64, 65]. As for the duration of treatment, a recent report confirmed that 1 month of treatment with atorvastatin 80 mg was sufficient to inhibit the pro-inflammatory transcription factor NF-κB and significantly decrease blood inflammatory mediators [66]. Choosing hepatitis A for vaccination allowed to directly assess the effect of atorvastatin on the immune response of seronegative individuals. The hepatitis A vaccine is indeed one of the few vaccines which results in a 98-99% seroconversion after a single vaccine dose in healthy individuals [53]. It is also known to be sensitive to immunomodulation, as indicated by a strong reduction of immunogenicity in HIV-1 infected patients [67, 68]. Finally, it is increasingly frequently recommended to travelers, including those under statin therapy.

Despite the influence of statins on the inflammatory response (inhibiting the expression of MHC Class II, activation of T-helper cells, and secretion of certain pro-inflammatory cytokines) and despite their positive influence on Th2-type lymphocyte responses, we did not observe any effect, negative or positive, from a short-term treatment with atorvastatin on the antibody response of healthy young individuals to hepatitis A virus vaccination. There was no increase or decrease in the primary end point, as reflected by the similar distribution patterns of serum antibody levels post vaccination. As expected, all participants who were on atorvastatin reduced their lipid as well as hsCRP values, confirming the rapid efficacy of this statin in reducing both lipid parameters and inflammation [12].

Interestingly, Pui Y. Lee and colleagues recently reported that a 10-day treatment with atorvastatin 40 mg induced a significant increase in the humoral response of young healthy volunteers receiving a tetanus toxoid booster on day 5 of

treatment [69]. However, contrary to our findings after a 1 month treatment with the same drug, acute-phase reactants, including CRP, did not change significantly in their study.

The potential limitations of our study design merit consideration. First, statin treatment was started on the same day as vaccination, so that the drug may not have exerted full anti-inflammatory and immunomodulatory actions during the process of antibody production. However, antibodies to hepatitis A vaccination appear in the serum between 2 to 4 weeks after immunization [70], allowing sufficient time for a potent immunomodulatory agent to exert its influence. Second, hepatitis A is a very strongly immunogenic vaccine which could better resist to subtle statin-induced variations of immune responses than less potent immunogens. Both these points were addressed in a murine model in which BALB/c mice were injected with atorvastatin or placebo prior to and during 3 weeks after immunization with a combined diphtheria-tetanus-pertussis-polio-Hib-hepatitis B vaccine containing strong (tetanus) and weak (diphtheria, hepatitis B) antigens. In accordance with the observations reported here, atorvastatin treatment did not influence murine antibody responses to any of the antigens tested (our own unpublished observation). Third, the participants were healthy volunteers, without any disease or concomitant medication in contrary to most patients on statins. Whether statin treatment would impact the antibody responses of frail elderly to influenza immunization may thus not be directly extrapolated from this study.

In conclusion, it is highly important to underscore that treatment with a potent immunomodulatory statin did not induce a deleterious effect on seroconversion rates post vaccination. Our findings strongly suggest that statin therapy does not interfere with antibody production and may thus be safely continued during immunization.

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DISCLOSURES

The authors have no conflict of interest to declare.

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Table I

Baseline characteristics of the participants.

	Atorvastatin n= 67	Placebo n=56
Age (years)	24±5.1	23.8±4.5
Female (%)	54	55
BMI *	21.8±2.3	21.5±2.4
Current smoker (%)	37	38

Values are means ± standard deviations. None of the differences between the groups were statistically significant. *Body-mass index is defined as the weight in kilograms divided by the square of the height in meters.

Table II

Evolution of baseline total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride and high-sensitivity C-Reactive Protein levels after 4 weeks of treatment.

	Atorvastatin (n=67)		Placebo (n=56)	
	Baseline	Follow-up	Baseline	Follow-up
Total-c (mmol/L)	4.6±0.7	3.0±0.5*†	4.7±1.7	4.8±1.9
LDL-c (mmol/L)	2.8±0.5	1.4±0.2*†	2.8±1.5	3.0±1.9
HDL-c (mmol/L)	1.4±0.3	1.3±0.4	1.4±0.3	1.3±0.3
Triglycerids (mmol/L)	1.0±0.7	0.8±0.4*†	1.2±0.8	1.2±0.5
hsCRP (mg/L)	1.69±1.89	1.24±1.28*†	1.47±1.95	1.61±1.39

Values are means ± standard deviations. * $p<0.05$ between baseline and follow-up; † $p<0.05$ between groups at follow-up.

Figure 1

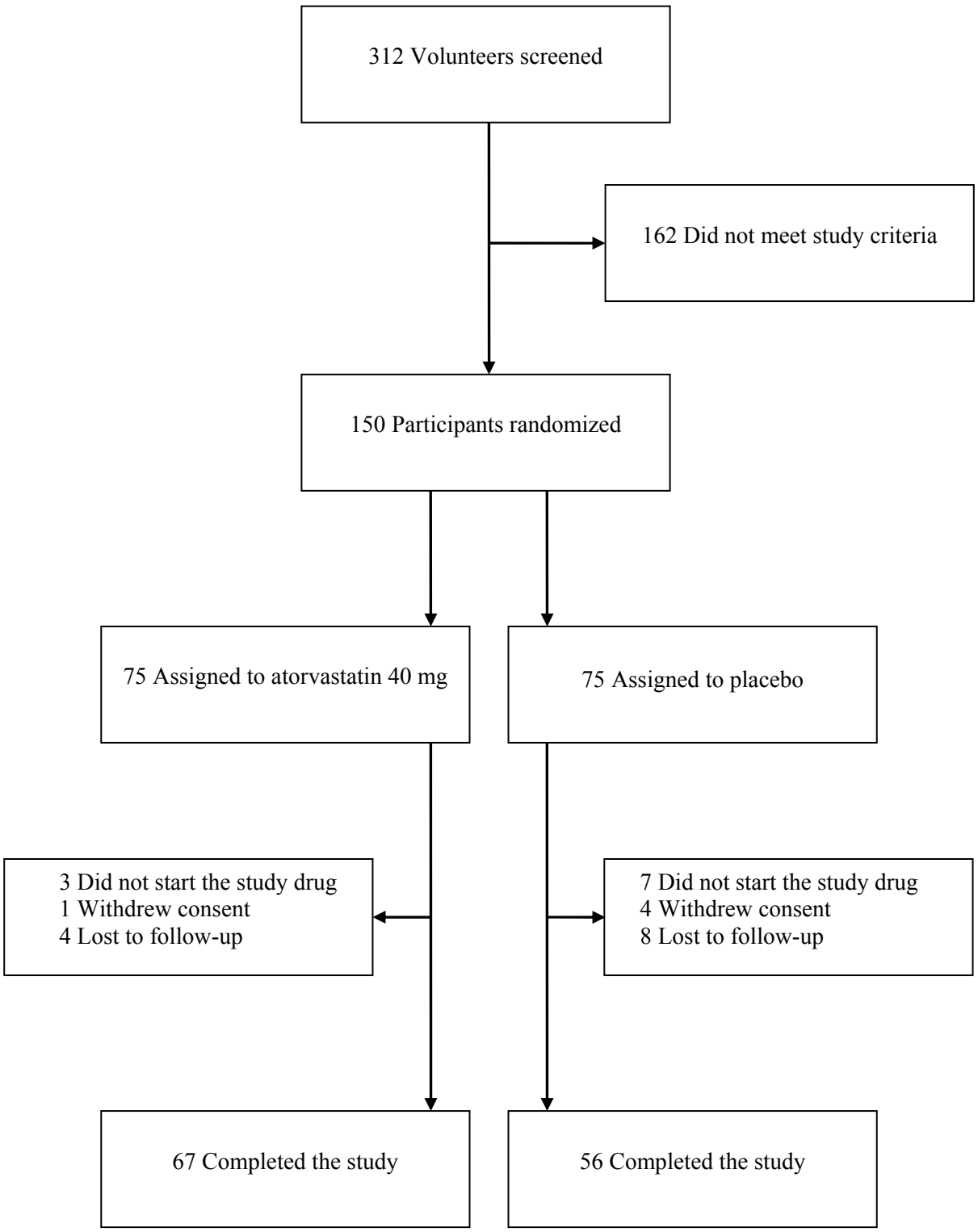
312 Volunteers screened
162 Did not meet study criteria
150 Participants randomized
75 Assigned to atorvastatin 40 mg
75 Assigned to placebo
3 Did not start the study drug
1 Withdrew consent
4 Lost to follow-up
7 Did not start the study drug
4 Withdrew consent
8 Lost to follow-up
67 Completed the study
56 Completed the study

Figure 2

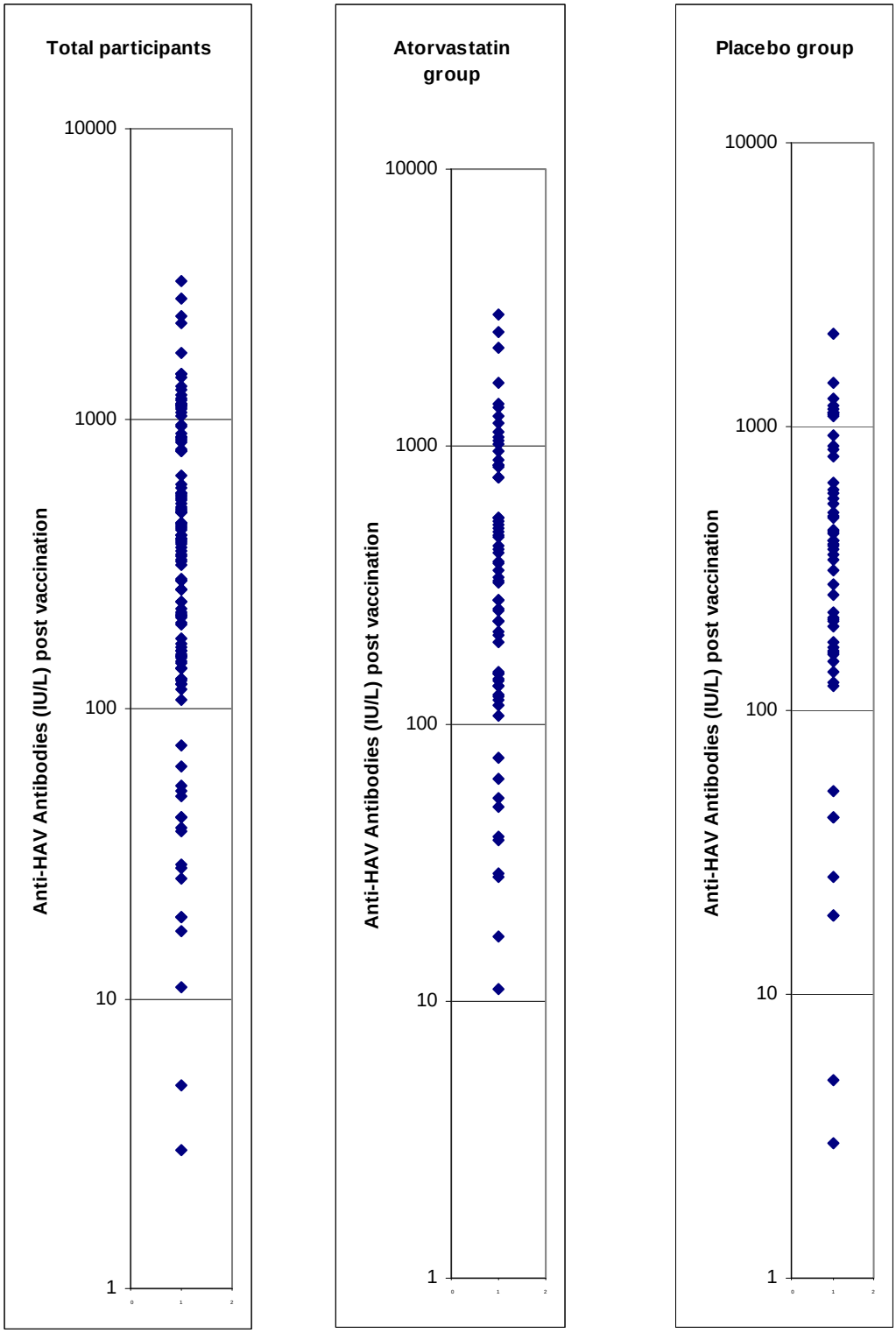
Total participants
Atorvastatin group
Placebo group
Anti-HAV Antibodies (IU/L) post vaccination
10000
1000
100
10
1

Figure 3

Immune response after hepatitis A vaccination according to quartiles of anti-hepatitis antibodies achieved.

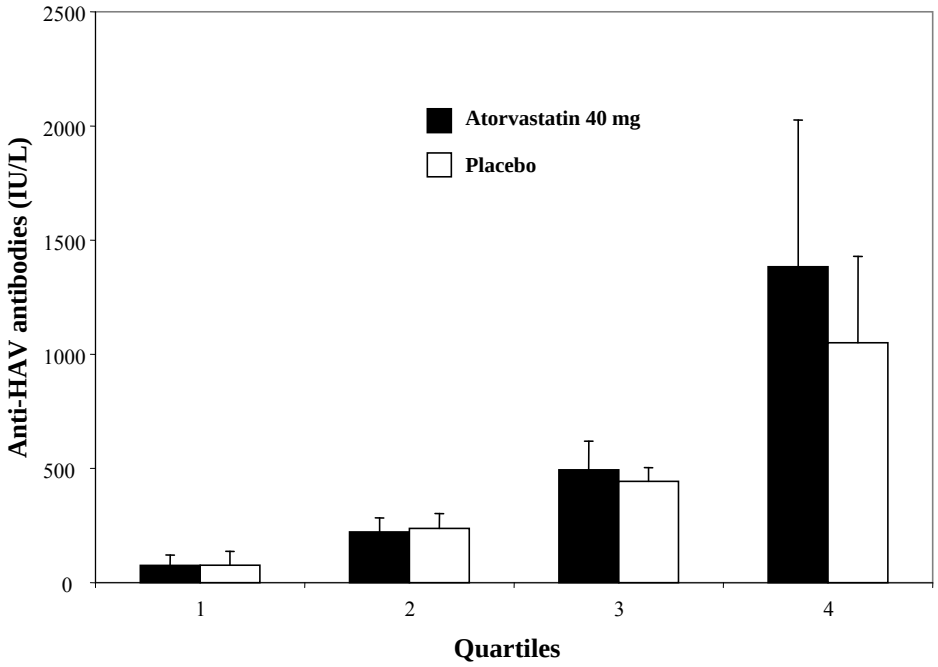

Figure Legends

Figure 1

Number of participants who were randomly assigned to a treatment group, who started the assigned treatment, and who discontinued treatment or were lost to follow-up. 89 % of participants in the atorvastatin group and 75 % in the placebo group completed the study.

Figure 2

Distribution of antibody levels after hepatitis A virus immunization.

Figure 3

Means and standard deviations of the antibody response by different quartiles after hepatitis A virus immunization.