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In vivo study of hepatitis B vaccine effects on inflammation and metabolism gene expression

Heyam Hamza · Jianhua Cao · Xinyun Li · Shuhong Zhao

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Abstract Pharmaceutical companies usually perform safety testing of vaccines, but all requirements of the World Health Organization and drug pharmacopoeias depend on general toxicity testing, and the gene expression study of hepatitis B vaccine is not done routinely to test vaccine quality. In this study, we applied a new technique of gene expression analysis to detect the inflammation and metabolism genes that might be affected by hepatitis B vaccine in mouse liver. Mice were used and divided into three groups: the first and second groups were treated with one or two human doses of vaccine, respectively, and the third group was used as a control. A microarray test showed that expression of 144 genes in the liver was significantly changed after 1 day of vaccination. Seven of these genes, which were related to inflammation and metabolism, were chosen and confirmed by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) at 1, 4 and 7 days. The expression level of these genes can be considered as a biomarker for the effects of the vaccine.

Keywords Hepatitis B vaccine · Inflammation genes · Metabolism genes · DNA microarray · Quantitative RT-PCR analysis

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H. Hamza · J. Cao · X. Li · S. Zhao (✉)
Key Lab of Animal Genetics, Breeding, and Reproduction of Ministry of Education, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China
e-mail: shzhao@mail.hzau.edu.cn

Introduction

Although vaccines are important pharmaceutical products that prevent infectious disease, their constituent chemicals and heavy metals can cause side effects. Aluminum is used as adjuvant to boost the immune response to vaccines. Recombinant adsorbed hepatitis B vaccine is prepared from transformed Chinese hamster ovary cells, and is a liquid product that contains hepatitis B surface antigen that is rendered insoluble by adding aluminum salt [1]. Immunization with the recombinant hepatitis B vaccine might produce antiphospholipid antibodies in genetically susceptible individuals, which might confer the risk of developing a continuous autoimmune response [2]. Hepatitis B vaccine administration can be followed by various rheumatic conditions and can trigger the onset of underlying inflammatory or autoimmune rheumatic diseases [3]. A previous study on the effect of adjuvant has shown that aluminum accumulates in the brain and results in neurodegeneration. The evidence for a link between aluminum neurotoxicity and Alzheimer's disease continues to grow stronger. Aluminum, like mercury, activates microglia, leading to chronic brain inflammation, which is a major event in both Alzheimer's disease and Parkinson's disease [4]. Flarend and co-workers have studied the fate of radiolabeled aluminum adjuvant, either aluminum hydroxide or aluminum phosphate, the two approved forms of adjuvant used in vaccines, and blood levels of aluminum remained elevated for 28 days with both adjuvants. Elevated aluminum levels were found in the kidney, spleen, liver, heart, lymph nodes and brain [5]. The purpose of safety or abnormal toxicity tests is to detect any toxicity of abnormal contaminants. One human dose of vaccine (no more than 1.0 ml) is injected intraperitoneally into each of five healthy mice that weigh 17–22 g, and the animals are

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observed for 7 days. The preparation passes the test if none of the animals shows signs of ill health. If more than one animal dies, the preparation fails the test. If one of the animals dies or shows signs of ill health, the test is repeated. The preparation passes the test if none of the animals in the second group dies or shows signs of ill health within the time interval specified [6]. The World Health Organization supports the replacement, reduction and refinement of the use of in vivo methods for biological preparation control and regularly conducts reviews of its recommended procedures for biological preparations to reduce the use of animals [7].

A new method has recently been developed that depends on measuring the body weight changes in addition to histopathological and hematological analysis, to test the quality and safety of different types of vaccine, including hepatitis B vaccine [8]. Development of gene expression analysis and the widespread use of such techniques in toxicology studies have enabled researchers to evaluate the safety of pertussis vaccine, which affects expression of many genes in several organs in vaccine-treated animals. In particular, the lungs appear to be the most suitable target to evaluate pertussis vaccine toxicity, and 13 genes have been identified in the lungs, which show a clear dendrogram that corresponds to vaccine toxicity. Furthermore, quantitative analysis of these genes has revealed a positive correlation between their expression levels and the degree of toxic effects [9]. Another study on pertussis vaccine has examined gene expression in relation to vaccine toxicity using a DNA microarray. Comparison of conventional animal test data with those from DNA-microarray-based gene expression has revealed an expression pattern that is highly correlated with leukocytosis in animals. Two genes, α1-acid-glycoprotein (Agp) and hemopexin (Hpx) have been found to be upregulated by vaccine administration. Variation in the gene expression was very small among the test animals, and the results were highly reproducible. These findings suggest that gene expression analysis of vaccine-treated animals can be used as an accurate and simple method for safety assessment of pertussis vaccine [10].

It has been shown that mouse liver damage induced by Bacillus Calmette-Guerin (BCG) vaccine and lipopolysaccharide (LPS) is highly correlated with immune reactions, cell synthesis, metabolism, apoptosis and transportation in liver cells, which might be important for elucidating the regulatory network of gene expression that is associated with liver damage [11]. DNA microarray analysis can be used for quality control of pandemic and endemic influenza virus vaccines. Hierarchical clustering of each influenza vaccine by the expression profiles of the 76 genes matched data from current quality control tests in Japan, such as the abnormal toxicity test (ATT) and the leucopenia toxicity test (LTT). Thus, it can be concluded that DNA microarray technology is an informative, rapid and highly sensitive method with which to evaluate the quality of influenza vaccines. The results using DNA microarray systems are consistent with those from ATTs and LTTs [12].

In this study, we tried to identify the effects of vaccine on inflammation and metabolism genes using DNA microarray analysis and quantitative PCR (q-PCR) to develop a gene expression detection system that can be used to study the in vivo effects of vaccine.

Materials and methods

Vaccines

Recombinant hepatitis B vaccine from Chinese hamster ovary cells (CHO) was manufactured by Huabei Medicine (China) and purchased from the Institute of Non-communicable Disease-Wuhan.

Animals

Male Kunming mice, aged 4 weeks and weighting 17–20 g, were used in the experiment. The temperature was maintained between 24 and 26°C.

Mice were divided into three groups: the first group was injected intraperitoneally with 20 μg/ml (one human dose) of hepatitis B vaccine; the second group was injected with 40 μg/2 ml (two human doses) of vaccine; and the third group was considered as control mice.

RNA preparation

Vaccinated mice were sacrificed at 1, 4 and 7 days, with three mice being killed at each time point. The liver was removed, immediately frozen in liquid nitrogen, and stored at −70°C. Total RNA was extracted by the TRIzol method, and checked for quantity and purity with an (Thermo scientific, USA) and integrity by gel electrophoresis.

RT-PCR

According to the manufacturing method of Ferments the first cDNA was prepared by 2 μg RNA. The first step prepared by mixing 2 μg RNA with 1 μl oligo dt primer and making the volume up to 12 μl with the water, followed by mixing, centrifugation and incubation at 65°C for 5 min. In the second step 4 μl buffer, 1 μl RNase inhibitor, 2 μl dNTP mix and 1 μl reverse transcriptase were added to yield a total volume of 20 μl followed by mixing, centrifugation and incubation at 42°C for 60 min, finally the sample was incubated at 70°C for 5 min and stored at −70°C.

Table 1 Sequences and annealing temperatures of primers

Gene	Annealing temperature	Forward primer	Reverse primer
Acaala	63°C	5′-TGGAAACTCCAGTCAGGTGAGTGAT-3′	5′-TCCTAGCTTCTCCACACAGTAGACG-3′
Acaalb	59°C	5′-GATTCCTATGGGGATAACTTCG-3	5′-ATGGTTTTCTTGTCACCCTTGT-3′
Saa2	63°C	5′-AGCCTGGTCTTCTGCTCCCT-3′	5′-GGTGTCCTCGTGTCCTCTGC-3′
Pck1	63°C	5′-CAAGACAGTCATCATCACCCAAGAG-3′	5′-TAGGGCGAGTCTGTCAGTTCAATAC-3′
Fgl1	60°C	5′-TGATGATGGGAAGGGAAGGT-3′	5′-CAGGCTCTGAAGAGGTTTGATT-3′
S100A8	62°C	5′- GCAACCTCATTGATGTCTACCA-3′	5′- GAGATGCCACACCCACTTTTAT-3′
S100A9	60°C	5′- GCATAACCACCATCATCGACAC-3′	5′CCATCAGCATCATACACTCCTC-3′
GAPDH		5′-TCACCATCTTCCAGGAGCGA-3	5′-GGCAGAAGGGGCGGAGATGA

DNA microarray analysis

The RNA labeling and hybridization were conducted by a commercial Affymetrix array service (GeneTech Biotechnology, Shang Hai, China). A total of 5 μg RNA was converted to double-stranded cDNA using the one-cycle cDNA Synthesis Kit (Affymetrix, Santa Clara, CA, USA) and an oligo-dT primer that contained the T7 RNA polymerase promoter. In vitro transcription of cRNA from cDNA was conducted using the MEGAscript T7 Kit (Ambion-USA) cDNA and cRNA were purified using the Sample Cleanup Module (Affymetrix). The GeneChip IVT Labeling Kit (Affymetrix) was used for synthesis of biotin-labeled cRNA. cRNA quality and concentration was checked by ultraviolet (UV) spectrophotometry. Two micrograms cRNA was then checked by formaldehyde denaturing gel electrophoresis in 1.2% agarose gel characterized by dispersed straps (28S and 18S) without any obvious smearing patterns from degradation. Subsequently labeled cRNA was fractionated and hybridized with the GeneChip Mouse Genome Array, according to manufacturer's instructions. Chips were washed and stained with a GeneChip Fluidics Station 450 (Affymetrix) using the standard fluidics protocol. The probe arrays were scanned using the Affymetrix GeneChip Scanner 3000. Six microarrays were used in the experiment, which corresponded to RNA from the liver tissue of three vaccinated mice and three controls. We used commercial software, Partek GS 6.5, for data comparison and analysis. The specific data analysis procedures were as follows. (1)The CEL files were imported in accordance with Robust Multi-array Analysis (RMA) of Partek GC6.5 software, to obtain RMA data. (2) Principal component analysis (PCA) of cluster analysis (3) According to the sample group, between groups multivariate analysis of variance statistical analysis (ANOVA) was used. (4) $P \leq 0.05$ was considered to be significantly different.

RMA analysis methods included the following four major steps: background correction on the perfect-match (PM) values; quantile normalization across all the chips in the experiment; Log2 transformation and Median polish summarization. [13].

Q-PCR

Expression of genes that were used as biomarkers for inflammation and metabolism changes was detected by q-PCR. First-strand cDNA was prepared by RT-PCR and diluted 5-fold from 20 to 100 μl. 2 μl of cDNA was mixed with 10 ml of Sybergreen reagent. (Applied Science-Germany), 0.4 μl of each forward and reverse primer was used in a total volume 20 μl, according to the manufacturer's instructions of (Applied Science-Germany). q-PCR was performed using (Roche LightCycler 480-Germany) as follows: initiation at 95°C for 2 min; denaturation at 95°C for 20 s; annealing at different temperatures according to each primer sequence (Table 1); and final extension at 72°C for 5 min. The expression level of all genes was relative to that of mouse Glyseraldehyde-3-phosphate dehydrogenase (GAPDH). Data were analyzed by the excel method to calculate the cycle threshold (ΔCT) and calculate P values, $P < 0.05$ was considered significant.

Results

Identification of differentially expressed (DE) genes by microarray analysis in mouse liver following hepatitis B vaccination

The global gene expression profile in the liver of mice at 1 day after challenge with one dose of hepatitis B vaccine was compared with that of the control group. After statistical analysis, 144 transcripts were identified at $P < 0.05$. Top20 target genes are shown in (Table 2) and the total 144 genes are found in (Supplementary Table 1). Among these 144 genes, 52 were downregulated and 92 were upregulated. Biomarkers in molecular toxicology are often the gene expression products that are associated with

Table 2 Top 20 target genes at 1 day after administration of a single dose of hepatitis B vaccine, examined by microarray analysis are shown fold change (FC) and P value ($P < 0.05$)

Gene description	Gene ID	Log FC[a]	P-value
Serum amyloid A 2	NM_011314	−6.9651	4.63538E−05
Orosomucoid 2	NM_011016	−6.4863	1.82E−05
Serum amyloid A 1	NM_009117	−5.6516	0.000786769
serum amyloid A 3	NM_011315	−5.2574	0.000104971
Alpha-2-macroglobulin	NM_175628	−4.4732	4.69E−06
Proteinase 3	NM_011178	−4.4271	0.001764889
Tissue inhibitor of metalloproteinase 1	NM_001044384	−3.5893	0.001451174
Orosomucoid 3	NM_013623	−1.9983	0.0017
Fibrinogen-like protein 1	NM_145594	−1.60683	0.0174
S100 calcium binding protein A8 (calgranulin A)	NM_013650	−2.52025	0.0068
S100 calcium binding protein A9 (calgranulin B)	NM_009114	−2.39604	0.01669029
D site albumin promoter binding protein	NM_016974	−3.8015	0.001171899
stearoyl-coenzyme A desaturase 1	NM_009127	1.91193	0.0013
phosphoenolpyruvate carboxykinase 1, cytosolic	NM_011044	−1.4856	0.0021
acetyl-coenzyme A acyltransferase 1A	NM_130864	1.3614	0.0008
acetyl-coenzyme A acyltransferase 1B	NM_146230	1.16473	0.0002
Cytochrome P450, family 51	NM_020010	−1.1208	0.001
P450 (cytochrome) oxidoreductase	NM_008898	−0.93757	0.013
Cytochrome P450, family 7, subfamily a, polypeptide 1	NM_007824	−1.38745	0.0329
Metallothionein 2	NM_008630	−4.4812	7.34314E−05

[a] Log fc expression change (−) up regulated gene expression, (+) down regulated

health, disease and toxicity. Liver injury involves many biological processes, such as necrosis, inflammation and cholestasis. We selected seven genes according to their functions as a biomarker for liver injury from the day 1 data and evaluated them by q-PCR. We selected these biomarkers of inflammation as a complex set of biological processes that occur during liver damage. In addition to inflammation genes we also studied metabolism genes, especially those related to bile acid biosynthesis.

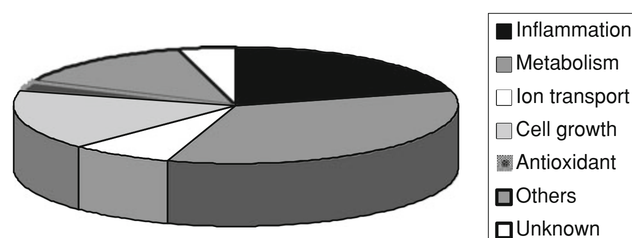

Fig. 1 Classification of 144 genes according to their functions

Quantification of inflammation and metabolism genes

In the cluster analysis of the liver, different genes were extracted on day 1 after one dose of hepatitis B vaccine. Top20 target genes are shown in (Table 2) and the total 144 genes are found in (Supplementary Table 1). Different gene functions appeared to change significantly (Fig. 1). We selected seven of the 144 genes whose functions were related to inflammation and metabolism (Table 3). This included upregulated genes S100calcium binding protein A9 (S100A9or calgranulin B), S100 calcium binding protein A8 (S100A8 or calgranulin A), serum amyloid A2 (Saa2), fibrinogen-like-protein 1 (Fgl1) and phosphoenol pyruvate carboxykinase1 (Pck1), and downregulated genes acetyl-coenzyme A acyltransferase 1A (Acaa1a) and acetyl-coenzyme A acyltransferase 1B (Acaa1b). We selected these genes according to their functions; Saa2 and Fgl1 are acute phase proteins that serve as biological markers for systemic inflammation. S100A8 and S100A9 also are considered as potentially useful clinical inflammatory markers and have been used as toxicity markers in a previous study on pertussis vaccine. We studied Acaa1a and Acaa1b because their functions are related to bile acid biosynthesis. Pck1 has an important role in liver gluconeogenesis. These seven genes included some that had a high fold change in expression, such as the upregulated gene Saa2 (−6.9651) and the down regulated genes Acca1a (1.3614) and Acca1b (1.16473),as appeared in the cluster analysis on day 1(Table 2) to follow their expression level on day 7.

Q-PCR results correlated well with microarray analysis and both methods showed significant changes in expression for all seven genes. To study the hepatitis B vaccine dose

Table 3 Four inflammation- and three metabolism-related genes were selected as biomarkers and confirmed by QPCR

Gene symbol	Gene ID	Category
S100A8	NM_013650	Inflammation
S100A9	NM_009114	Inflammation
Fgl1	NM_145594	inflammation
Saa2	NM_011314	inflammation
Pck1	NM_011044	Metabolism
Acaa1a	NM_130864	Metabolism
Acaa1b	NM_146230	Metabolism

effect on inflammation and metabolism genes, we used two q-PCR experiments. In the first of these, mice were challenged with one dose of vaccine, and in the second, mice were challenged with two doses (Figs. 2, 3). The effect of vaccine was observed at 1, 4 and 7 days. The expression level of S100A8 was significantly increased in the group with two doses of vaccine at all three time points. In the group with one dose of vaccine, S100A8 expression was only significantly increased at 1 and 4 days. This means that the vaccine had a prolonged effect when two doses were administered. Similar results were obtained for Saa2, S100A9 and Acaa1b which were significantly increased at all three time points in second group, whereas Fgl1, Acaa1a and Pck1 were only significantly increased at 1 day in both groups.

Discussion

Hepatitis B vaccine is similar to other pharmaceutical preparations that have local and systemic side effects; the most frequent local side effects that may occur include induration, pain and pruritus. The systemic side effects that persist or become troublesome when using recombinant hepatitis B vaccine include diarrhea, dizziness, fatigue, headache, irritability, loss of appetite and mild fever. The most harmful effects of vaccines are caused by their chemical constituents, such as aluminum that is used as an adjuvant in hepatitis B vaccine. We used gene expression analysis techniques of microarray analysis and q-PCR to study vaccine effects on inflammation and metabolism genes in the liver. These methods are more efficient than the conventional methods.

For the microarray method, we compared the liver from control and vaccinated mice that were challenged with one dose of hepatitis B vaccine and sacrificed after 1 day. We selected 1 day after vaccination to observe the initial effects of vaccine on gene expression. We studied the liver

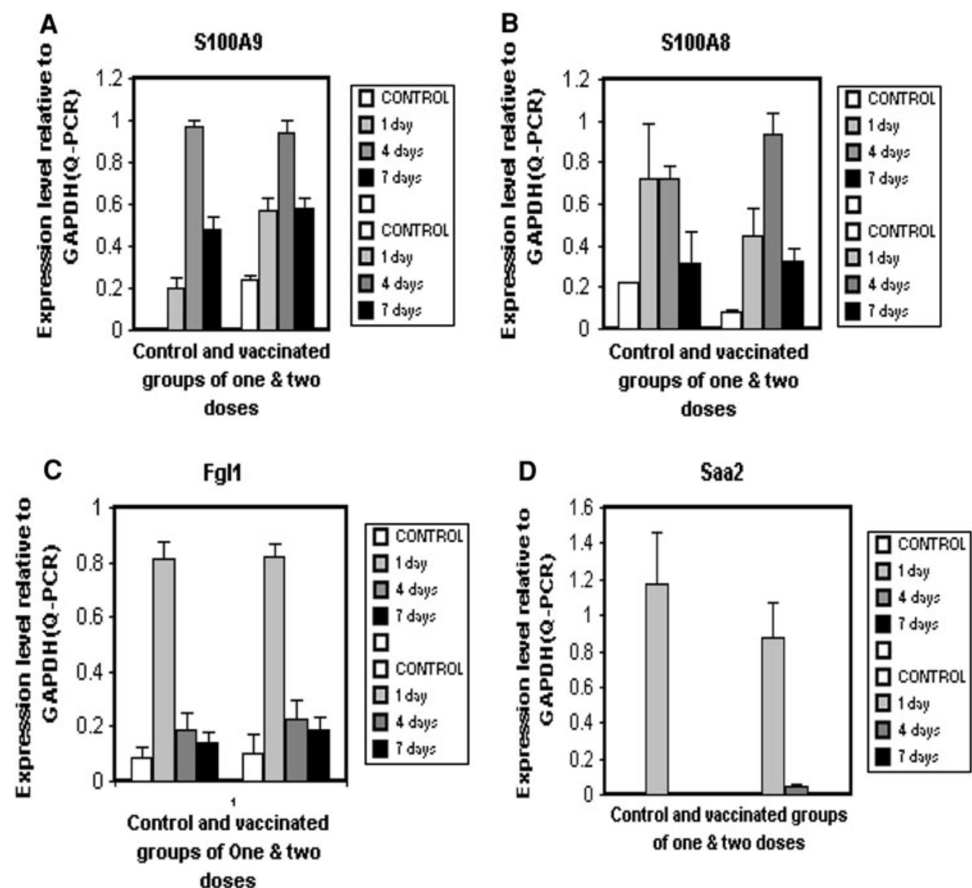

Fig. 2 Quantitative evaluation of genes by Q-PCR. Quantification data for four inflammation genes **a** (S100A9), **b** (S100A8), **c** (Fgl1) and **d** (Saa2) at 1, 4 and 7 days after one or two doses of hepatitis B vaccine. The data from three mice at each time point are shown as the mean ± SD. Data were assessed relative to GAPDH (P value < 0.05). Effect of 1 and 2 doses vaccine on expression level of inflammation genes

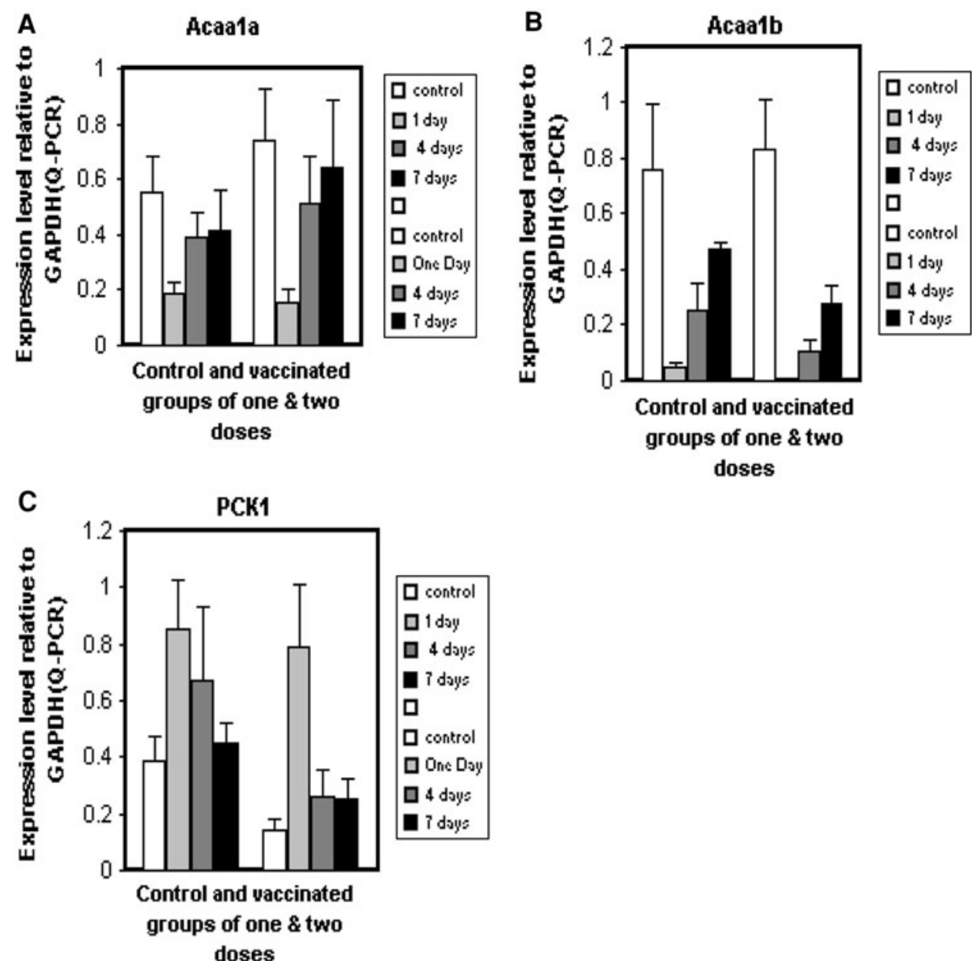

Fig. 3 Quantitative evaluation of genes by Q-PCR. Quantification data for three metabolism genes **a** (Acca1a), **b** (Acca1b) and **c** (Pck1) at 1, 4 and 7 days after one or two doses of hepatitis B vaccine. The data from three mice at each time point are shown as the mean ± SD. Data were assessed relative to GAPDH (*P* value < 0.05). Effect of 1 and 2 doses vaccine on expression level of metabolism genes

because it is the major organ for metabolism and excretion of toxic chemicals.

Other studies have investigated the toxic effects of synthetic materials and vaccines in the liver [10–12, 14, 15]. Hepatitis B vaccine has a broad spectrum of activity, and 144 genes with different functions, including metabolism and inflammation, were differentially expressed after vaccination. These results are similar to other studies on pertussis [9, 10]. BCG [11] and influenza [12] vaccines. Biomarkers in molecular toxicology are often the gene expression products that are associated with health, disease and toxicity. Liver injury involves many biological processes, such as necrosis, inflammation and cholestasis [16]. We selected seven genes according to their functions as biomarkers for liver injury from the day 1 data and evaluated them by q-PCR. We selected these genes as biomarkers of inflammation as a complex set of biological processes that occur during liver damage. In addition to inflammation genes, we also studied metabolism genes, especially those related to bile acid biosynthesis and its role in cholestasis.

Saa and Fgl1 are acute phase proteins that serve as biological markers for systemic inflammation and a result of liver injury, infection or neoplastic growth [17, 18]. Although the biological activity of acute phase proteins is mostly protective, they can also play roles in pathologic conditions. For example SAA proteins are often found in secondary amyloidosis [19]. S100A8 and S100A9 are also considered to be potentially useful clinical inflammatory markers [20], and have been used as toxicity markers in a previous study of pertussis vaccine [9]. The major harmful effect of vaccine is due to aluminum adjuvant and aluminum accumulation in the liver leads to pathophysiological damage, particularly bile duct cholestasis [21], therefore we studied Acaa1a and Acaa1b because they are involved in fatty acid metabolism and bile acid biosynthesis. We used the liver because it is a major metabolic organ and has been used in other studies on vaccines [9–12, 22].

We found that there were significant effects on the inflammation and metabolism genes on 1 day after hepatitis B vaccine administration (Table 2). Expression of inflammation-related genes, such as serum amyloid A (Saa1, Saa2, Saa3), orosomucoid (Orm2, Orm3), alpha-2-macroglobulin (A2 m), Fgl1, and calgranulin A and B suggests that acute inflammation is induced by vaccine treatment. In addition,

many forms of P450 cytochrome P450 (NM_020010), (NM_008898), (NM_007824) which are the major enzymes involved in drug metabolism are induced in the liver by vaccine treatment. Furthermore, the clustering data revealed significant changes in expression of metabolism genes such as Acaa1a and Acaa1b, stearoyl-coenzyme A desaturase 1(Scd1) and Pck1 at 1 day after vaccine administration. This suggests that the gene expression data on 1 day are sufficiently sensitive to detect vaccine effects. Day 1 was found to be the most reliable time point.

The data from the microarray analysis and q-PCR were shown to be reproducible. Our results also demonstrated that the liver might be the most suitable organ for detecting the effects of hepatitis B vaccine on inflammation and metabolism genes. The effects of two doses were shown to be prolonged compared with the effects of a single dose. Hepatitis B vaccine can affect acute phase genes such as Saa2, which was significantly increased at the three time points in the two-dose group compared with the single-dose group, which only had a significant increase after 1 and 4 days. The serum amyloid A (SAA) family of proteins consists of inducible acute-phase proteins and a constitutive member that are minor apolipoproteins of high-density lipoprotein (HDL) during inflammation, HDL cholesterol and apolipoprotein A-I (apoA-I) are decreased, and these changes are thought to be partly related to the increase in acute-phase SAA proteins that associated with HDL particle to become the major apolipoprotein species [23]. Infection and inflammation induce the acute-phase response which leads to multiple alterations in lipid and lipoprotein metabolism, Plasma triglyceride levels increase with very-low-density lipoprotein secretion that results from adipose tissue lipolysis, increased de novo hepatic fatty acid synthesis and suppression of fatty acid oxidation [24]. Several cytokines, first of all interlukin-1(IL-1), interlukin-6(IL-6) and Tumor necrosis factor-alpha (TNF) are involved in the induction of SAA synthesis [19]. SAA appears to be an important mediator of the inflammatory process. It might contribute to the pool of interlukin-8 (IL-8) that is produced in chronic diseases and might play a role in degenerative diseases [25] SAA is an acute-phase protein, whose level in the blood is elevated in response to trauma, infection, inflammation, and neoplasia [17]. SAA levels increase markedly during acute and chronic inflammation, and play a role in atherogenesis [26, 27].

Our results suggest that hepatitis B vaccine alters the expression of acute-phase proteins like Fibrinogen-like-protein 1 (Fgl1), which only significantly increased after 1 day with one or two doses of vaccine. Fgl1 is usually associated with acute inflammation. Fgl1 is a hepatocyte derived-protein that is upregulated in regenerating rodent livers following partial hepatectomy. It has been implicated as a mitogen for liver cell proliferation and it might serve as a diagnostic or prognostic biological marker in certain inflammatory conditions [18].

S100 calcium binding protein A8 (calgranulin A or S100A8) is a member of the S100 family of calcium-binding proteins that are chemotactic for myeloid cells, and it is expressed with its dimerization partner S100 calcium binding protein A9 (calgranulin B or S100A9) during myelopoiesis in the fetal liver [28]. These two genes were significantly increased at 1, 4 and 7 days following two doses of hepatitis B vaccine. In contrast, one dose of vaccine only increased at 1 and 4 days, it means the vaccine has prolonged effects after two doses, the same result that both of (S100A8, S100A9) are upregulated in the acute phase [29]. Calprotectin is a complex of two calcium-binding proteins that are abundant in the cytosolic fraction of neutrophils, and is present in the extracellular fluid during various inflammatory conditions, such as rheumatoid arthritis, cystic fibrosis and abscesses [30].

Fatty acid and bile acid synthesis in the liver was also affected by hepatitis B vaccine. This was demonstrated by the significant decrease in Acaa1a expression after 1 day with one or two doses of vaccine. The effect on Acaa1b appeared to be dose dependent. One dose of vaccine downregulated Acaa1b expression at 1 and 4 days, but two doses resulted in downregulation at 1, 4 and 7 days. Acaa1a and Acaa1b that are related to fatty acid metabolism and bile salts biosynthesis and are changed after exposure to Perfluorooctanoic acid (PFOA) [31].

Pck1 was significantly increased after 1 day by one or two doses of hepatitis B vaccine. Pck1 gene encoding cytosolic Phosphoenol pyruvate carboxykinase (PEPCK-C) plays a major role in the control of fatty acid release from adipose tissue [32]. Hepatotoxins such as pyrazole alter lipid and carbohydrate metabolism genes expression such as Pck1 [33]. Glucocorticoids affecting genes that are related to glucose metabolism, such as Pck1 [34]. Disregulated overexpression of PEPCK-C in adipose tissue increases fatty acid re-esterification that leads to obesity [35].

Conclusion

Although hepatitis B vaccine prevents infection, previous studies have shown that it has several side effects that are caused by the aluminum adjuvant. Therefore, we applied more sensitive and more scientifically well-grounded methods for the quality control of vaccine safety. We confirmed by quantitative RT-PCR that hepatitis B vaccine changed the expression level of seven genes that were selected as biomarkers, which reflected subtoxic/adverse effects of the vaccine, especially subtle liver injury. In addition to other genes that were significantly changed and

detected by microarray comparing with control to get the fold change data as a criteria was depended in other previous studies of vaccine safety by gene expression methods, This study tested the hypothesis that gene expression profiling can reveal indicators of subtle liver injury that is induced by a dose of a substance that does not cause overt toxicity as defined by conventional criteria of toxicology. Microarray does not replace the classical toxicity method but it can be used in pre-clinical development vaccine.

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