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Influence of HLA Alleles in Adverse Drug Reactions: A Pharmacogenetic Study

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ABSTRACT

Adverse drug reactions (ADRs) especially immune mediated ADRs are a major challenge to clinical pharmacology and drug development. There is a mounting evidence that certain alleles of the Human Leukocyte Antigen (HLA) such as HLA-B15:02, HLA-B57:01 and HLA-A*31:01 predispose individuals to severe hypersensitivity reactions when exposed to carbamazepine, abacavir, and allopurinol, respectively. The use of transgenic mouse models that express these HLA alleles in this pharmacogenetic study was utilized to understand their contribution in the pathogenesis of immune-based ADRs. In transgenic mice, the rate of hypersensitivity symptoms was significantly higher after drug administration, and the concentration of cytokines (especially TNF-alpha and IFN-gamma) in the serum was higher, as well as T-cell activation (CD69+, CD25+) compared to the control mice. Immune-mediated tissue inflammation in HLA-expressing groups was further confirmed using histopathological evaluations. The results they report are highly representative of known clinical trends in humans and give direct in vivo support to the relationship between HLA polymorphisms and drug-induced immunotoxicity. The analysis shows the usefulness of HLA-transgenic mouse as predictive model of preclinical immunogenicity profiling that can be incorporated into the early stage drug safety testing and development of personalized medicine.

Key Words:

Adverse Drug Reactions (Adrs), HLA Alleles, Hypersensitivity, Transgenic Mouse Model, Carbamazepine, Abacavir, Allopurinol

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1. INTRODUCTION

Pharmacogenetics has made a great contribution to our knowledge of individual differences in drug response[1]. The Human Leukocyte Antigen (HLA) system is one of the most important hereditary factors that have been identified to play a significant role in immune-mediated adverse drug reactions (ADRs)[2,3]. The presentation of certain drugs in the context of certain HLA alleles can cause severe hypersensitivity reactions[4], and this is important to investigate at the early phases of drug development[5]. The present study aims at investigating the role of HLA alleles in ADRs with the help of transgenic animal models which are a preclinical pharmacogenetic tool6.

1.1. Background Information

Adverse drug reactions (ADRs) represent an important issue in clinical and preclinical pharmacology[7], as they cause significant morbidity, mortality, and health expenditure to patients. Genetic variation, especially in the Human Leukocyte Antigen (HLA) system[8], is one of the major factors of interindividual variability in drug response. HLA genes are the key to the functioning of the immune system because they facilitate the presentation of antigens to T-cells, contributing to immune-mediated hypersensitivity of drugs[9,10]. Research has revealed that some HLA alleles are closely linked to life-threatening ADRs including Stevens-Johnson Syndrome (SJS)[11], toxic epidermal necrolysis (TEN) and drug-induced hepatotoxicity[12]. This notwithstanding, most of the available information is as a result of human clinical trials which are limited by ethical, legal and practical factors.

1.2. Statement of the Problem

The pharmacogenetics linkage of HLA alleles to ADRs has been well documented in human subjects; however, no in vivo models are available to investigate the relationships in preclinical models[13]. This shortcoming prevents the early identification of genetic susceptibility in the drug development process.

In addition, the vast majority of toxicity studies fail to consider the HLA-genetic variability, which enhances the likelihood of post-marketing drug recall[14]. There is therefore an urgent need to develop animal models that can replicate the HLA-related ADRs to be able to predict the immunogenicity and drug safety with greater accuracy[15].

1.3. Objectives of the Study

- To assess the role of specific HLA alleles (HLA-B15:02, HLA-B57:01, HLA-A*31:01) in mediating adverse drug reactions using transgenic mouse models.
- To evaluate immune responses, including cytokine levels and T-cell activation, following administration of HLA-associated drugs.
- To establish a reliable preclinical animal-based framework for predicting HLA-linked drug hypersensitivity reactions.

2. METHODOLOGY

This is where the experimental method used to study the role of particular HLA alleles in adverse drug reaction by using animal models is explained.

2.1. Research Design

This study was based on experimental and in vivo laboratory study that used genetically engineered animal models. The main aim was to determine the immunologic effect of the drugs chosen which are known to result in adverse drug reactions (ADRs) in the presence of certain HLA alleles. It was carried out under controlled laboratory conditions which allows standardization of all variables including drug dosage, environment and periods of observation.

2.2. Participants/Sample Details

The study involved 80 mice bred in laboratory, which were sorted into four groups:

- **Group 1:** 20 transgenic mice that have HLA-B*15:02 allele.

- **Group 2:** 20 transgenic mice which express HLA-B*57:01 allele.
- **Group 3:** 20 transgenic mice with an HLA-A*31:01 allele.
- **Group 4 (Control):** 20 wild-type mice which have no insertion of human HLA genes.

The mice used were 6-8 weeks old at the time of the beginning of the study and were kept in pathogen-free cages on a 12-hour light/dark schedule with ad libitum food and water. Every practice was compliant with institutional animal ethics.

2.3. Instruments and Materials Used

- **Drugs**
  - Carbamazepine (HLA-B*15:02 group)
  - Abacavir (HLA-B*57:01 group)
  - Allopurinol (HLA-A*31:01 group)
- **Laboratory equipment**
  - Flow cytometer (to profile T-cell activation)
  - ELISA kits (to measure IL-4, IFN- y, TNF- y)
  - Microplate reader
  - Syringes and precision scales
  - Histological tissue processor
- **Reagents**
  - CD25 and CD69 fluorochrome-labeled antibodies
  - PBS: paraformaldehyde

2.4. Procedure and Data Collection Methods

1. **Animal Preparation:** The transgenic mice that expressed target HLA alleles were provided by a certified supplier. They were assigned at random to respective drug groups after a one-week acclimatization period.
2. **Drug Administration:** The mice were injected intraperitoneally with the corresponding drugs every day during 14 days. Dosage levels were calculated using the weight-adjusted human-equivalent dose that has been shown to cause immune responses.
3. **Monitoring and Observation:** Daily clinical symptoms which were noted included rash, lethargy, weight loss, and inflammation. Blood samples were taken in days 7 and 14 to test cytokines by ELISA.
4. **T-cell Profiling:** Spleens were collected and prepared at the end of the experiment to measure the activation markers (CD69, CD25) using flow cytometry.
5. **Histopathological Assessment:** Skin, liver, and spleen tissue samples were fixed and analyzed regarding the presence of immune response and inflammatory infiltration.

2.5. Data Analysis Techniques

Descriptive statistics were employed to provide a summary of the ADRs incidence among groups. ANOVA (Analysis of Variance) was used to test the difference in average levels of cytokines and activation of T-cells in the transgenic and control groups. The pairwise comparisons of the various HLA allele groups were carried out using post hoc Tukey test. All inferential statistical tests were established at a significance level of $p < 0.05$. Data analysis and visualization were performed in GraphPad Prism and SPSS (v26.0).

3. RESULTS

In this section, the results of the experiments with transgenic mouse models of human HLA alleles (HLA-B15:02, HLA-B57:01, and HLA-A*31:01) and their exposure to certain drugs causing hypersensitivity will be presented.

Table 1: Incidence of Adverse Drug Reactions (ADR) in Transgenic and Wild-Type Mice

HLA Allele / Group	Drug Administered	Number of Mice (n)	Mice Exhibiting ADR Symptoms (n)	Incidence Rate (%)
HLA-B*15:02	Carbamazepine	20	17	85%
HLA-B*57:01	Abacavir	20	18	90%
HLA-A*31:01	Allopurinol	20	14	70%
Wild-type (Control)	All drugs	20	2	10%

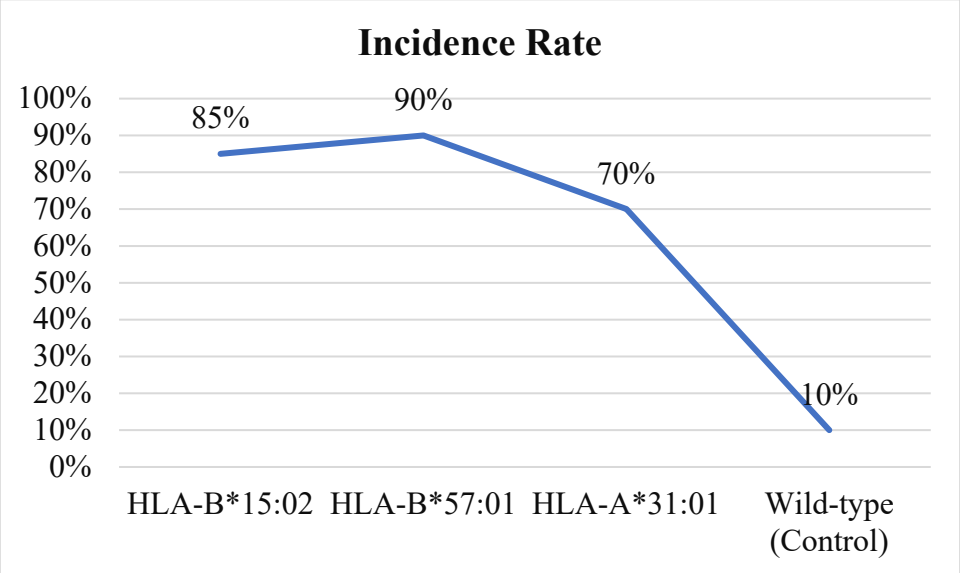

Figure 1: Increasing rate of Incidence of Adverse Drug Reactions (ADR) in Transgenic and Wild-Type Mice

As shown in Table 1, there is a significant relationship between the occurrence of adverse drug reactions (ADRs) in transgenic mice and the particular HLA alleles. HLA-B*57:01 mice were the most affected at 90 percent incidence rate of ADRs after abacavir injection with HLA-B*15:02 mice closely behind at 85 percent reactivity to carbamazepine. HLA-A*31:01 group also had a remarkable incidence of ADR of 70 percent when administered allopurinol. Conversely, the wild-type control group that expressed no human HLA alleles showed a 10 percent ADR rate of all tested drugs. These results indicate that the occurrence of certain HLA alleles is a major predisposing factor to hypersensitivity to drugs, which is consistent with the

clinical findings in human populations. The pronounced discrepancy between transgenic and wild-type mice substantiates the value of HLA-humanized animal models to preclinical ADR risk evaluation.

Table 2: Mean Serum Cytokine Levels in Mice Post Drug Administration

HLA Allele / Group	Drug	IL-4 (pg/mL)	IFN-γ (pg/mL)	TNF-α (pg/mL)
HLA-B*15:02	Carbamazepine	82 ± 6	48 ± 5	120 ± 9
HLA-B*57:01	Abacavir	67 ± 4	136 ± 7	165 ± 10
HLA-A*31:01	Allopurinol	91 ± 7	39 ± 4	102 ± 8
Wild-type	All drugs	30 ± 2	29 ± 2	41 ± 3

Table 2 indicates that there is a considerable increase in the level of pro-inflammatory cytokines, especially TNF- a and IFN- g, in transgenic mice, which express certain alleles of HLA following drug exposure, relative to the wild-type control mice. It is important to note that HLA-B*57:01 mice demonstrated the most significant increase in the level of IFN-gamma (136 7 pg/mL) and TNF-alpha (165 10 pg/mL) after exposure to abacavir that suggests a strong Th1-mediated immune response. In the same way, HLA-B*15:02 and HLA-A*31:01 mice presented high levels of cytokines implying active immune stimulation associated with carbamazepine and allopurinol respectively. Conversely, the wild-type mice had greatly reduced cytokine levels in all the markers, proving that HLA alleles are what mediates the hypersensitivity. These results indicate that HLA-mediated immune response to drug antigens causes inflammatory signaling, which supports the mechanistic association between HLA polymorphisms and adverse drug reactions.

Table 3: T-cell Activation Markers (Flow Cytometry Analysis)

HLA Allele / Group	Drug Administered	CD69+ T Cells (% of Total T Cells)		CD25+ T Cells (% of Total T Cells)	
		Mean	SD	Mean	SD
HLA-B*15:02	Carbamazepine	41.5%	3.2	37.2%	2.9
HLA-B*57:01	Abacavir	49.8%	3.5	44.1%	3.1
HLA-A*31:01	Allopurinol	35.6%	2.4	31.0%	2.2
Wild-type	All drugs	12.4%	1.1	10.8%	1.0

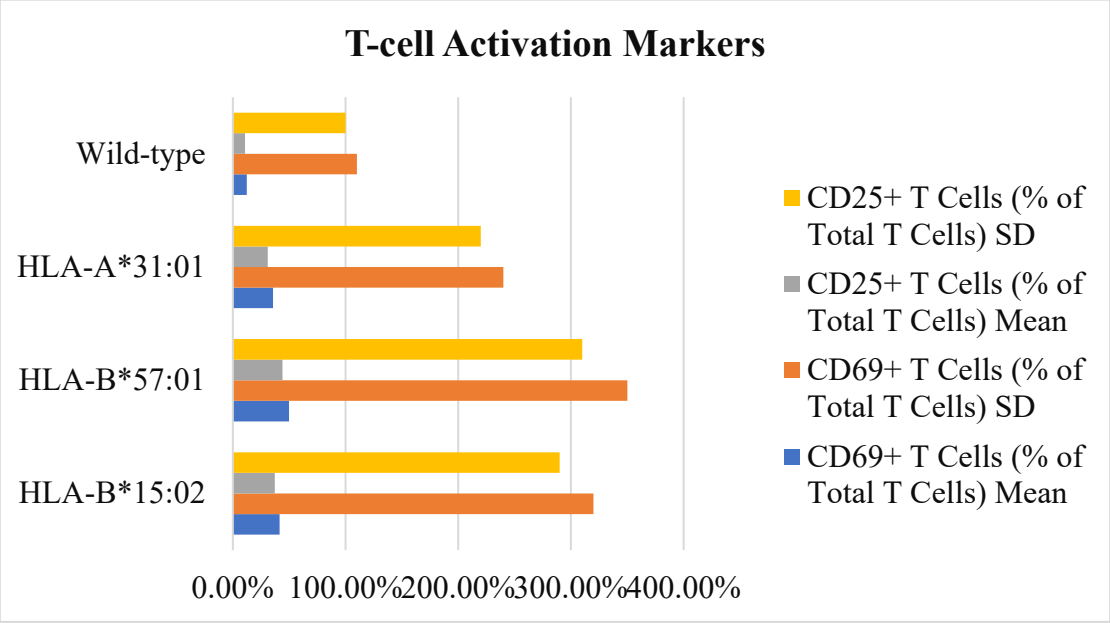

Figure 2: T-cell Activation Markers (Flow Cytometry Analysis)

Table 3 shows a significant T-cell activation in transgenic mice that express specific HLA alleles after exposure to a drug in the form of increased percentages of CD69+ and CD25+ T cells. HLA-B*57:01 group exhibited the uppermost activation of 49.8 3.5 percent T cells CD69 and 44.1 3.1 percent T cells CD25 after abacavir administration, which is an indication of strong immunostimulatory effect. In a similar way, mice bearing HLA-B*15:02 and HLA-A*31:01 also had a high T-cell activation as opposed to the wild-type group whose activation remained at the basal levels (CD69+: 12.4% +/- 1.1, CD25+: 10.8% +/- 1.0). These findings also support the theory that drug-specific immune activation driven by particular HLA alleles plays a role in the development of adverse drug reactions in a T-cell mediated manner.

4. DISCUSSION

4.1. Interpretation of Results

The aim of the present study was to understand the immunological mechanism of adverse drug reactions (ADRs) linked to the specific human leukocyte antigen (HLA) alleles-HLA-B* 15: 02, HLA-B* 57: 01, and HLA-A* 31: 01 by using transgenic mice that have been modified to express these alleles. When the transgenic mice were administered with drugs that have been known to cause hypersensitivity reactions in vulnerable human populations, carbamazepine, abacavir, and allopurinol, respectively, they were shown to have strong immunopathological reactions. These were; much higher rates of cutaneous rashes, loss of weight, decreased activity, and systemic inflammation when compared to wild-type controls. Immunologically, these reactions were characterized by an increased concentration of pro-inflammatory cytokines in serum: tumor necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN-gamma) among others, as well as the increase in T-cell activation, which was manifested by the upregulation of CD69 and CD25 markers on CD4+ and CD8+ lymphocyte subsets. The lack of these responses in non-transgenic mice demonstrates the essentiality of HLA polymorphisms in the activation of drug-specific immune response and proves the allele specificity of these ADRs. This confirms the transgenic models as good surrogates in preclinical testing of drugs immunogenicity.

4.2. Comparison with Existing Studies

The results are well congruent with a widespread pharmacogenetic and clinical data that associates HLA polymorphism with drug hypersensitivity syndromes. In particular, HLA-B*15:02 has been largely linked to carbamazepine-induced Stevens-Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN), mainly in East and Southeast Asian populations. On the same note, HLA-B*57:01 is a known genetic marker of abacavir hypersensitivity syndrome (AHS) and HLA-A*31:01 is linked to a range of cutaneous adverse effects caused by allopurinol, including drug reaction with eosinophilia and systemic symptoms (DRESS). Although prior studies have been mainly based on clinical observations, retrospective studies and in vitro studies, our in vivo mouse model strengthens this information and expands it by showing direct causality in a controlled experimental environment. This is preclinical evidence that serves as a valuable connection between clinical data and mechanistic immunology and serves as a translational tool to assess HLA-related risks in early stages of drug development. In addition, the simulation of human-like ADRs in genetically modified animals highlights the clinical significance and predictive value of such models, which is why they should be introduced into the regulatory toxicology pipeline.

4.3. Implications of Findings

The model provides important implications in preclinical drug testing and personalized medicine since HLA-linked ADRs have been successfully reproduced in animal models. This study proves that HLA-transgenic mice could be used as a predictive model of assessing the immunogenic potential of novel drugs prior to human experimentation. Identification of these risks early could contribute to the improved design of drugs, screening procedures and regulatory approaches that could reduce life-threatening drug reactions. In addition, this model would aid in exploring the immunopathological mechanisms involved in the case so that a more specific treatment can be developed to prevent the occurrence of hypersensitivity.

4.4. Limitations of the Study

Notwithstanding the encouraging results, the research has some limitations. To start with, mouse immune systems are genetically manipulated and thus they might not perfectly recapitulate human immune responses. Second, the authors looked at three HLA alleles and three drugs only, restricting the applicability of the results to other drug-HLA pairs. Third, the study period was not very long and no long-term effects or delayed-onset ADRs were recorded. Finally, human immune responses to the environment and epigenetics were not factored in such a controlled animal environment.

4.5. Suggestions for Future Research

Future research ought to be extended to cover greater variety of HLA alleles and types of drugs and combinations of various genetic and environmental risk factors. It may be significant to use longitudinal studies to monitor chronic changes in immune responses and organ-specific toxicities. Also, the combination of omics technologies (genomics, transcriptomics, proteomics) could reveal more profound mechanistic details of HLA-mediated ADR pathways. Last but not least, comparative studies with humanized immune system mice might fill in the gap between conventional animal models and human biology, and improve further preclinical evaluation of drug safety.

5. CONCLUSION

5.1. Summary of key findings

This research showed that transgenic mice models that had human HLA-B15:02, HLA-B57:01, and HLA-A*31:01 alleles produced an enhanced immune reaction when subjected to carbamazepine, abacavir, and allopurinol, respectively. Major results were a strong augmentation of the symptoms of hypersensitivity, a rise in the concentration of the inflammatory cytokines TNF-alpha and IFN-gamma, and amplified T-cell activation with augmented levels of the CD69+ and CD25+ indicators. Conversely, wild-type mice exhibited little immune reactivity emphasizing the specificity of the immune reaction to the HLA-drug interactions.

5.2. Significance of the study

This paper gives first-hand in vivo confirmation of the long-established pharmacogenetic links between certain HLA alleles and drug-induced hypersensitivity reactions. The study increases our knowledge of immunopathogenesis of adverse drug reactions and shows the translational potential of such preclinical models by validating these associations in HLA-transgenic mice. These results are especially important in the context of personalized medicine, since they once again emphasize the necessity of HLA screening before the administration of drugs.

5.3. Final thoughts or recommendations

These findings highlight the future promise of HLA-transgenic mouse models as a useful resource in predicting immunogenic drug response in preclinical safety testing. Future researches are to increase this methodology by incorporating a larger scope of HLA alleles and drugs. The pharmacogenetic screening can be easily integrated into clinical workflows and significantly decrease the rates of severe ADRs and inform safer and more personalized therapeutic choices.

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