

Editorial

Immune hemolytic anemia and the chemical reactivity of drugs

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Introduction

Drug-induced immune hemolytic anemia (DIHA) is rare but potentially fatal. Its incidence has been estimated to be around one in one to two million patients. Red blood cells are marked with drug-induced antibodies and destroyed either intravascularly by complement, or extravascularly, for example inside the spleen. The only effective therapy is to discontinue the offending drug.^{1,2}

As of 2012, around 138 drugs or drug-like substances are proven to have caused DIHA. The most important drugs include cephalosporins, especially of the third generation, such as cefotetan. Currently, a penicillin - piperacillin - is the major cause of DIHA, with 32 cases described within a ten-year-period (2003 - 2012) by the group of the late Dr. George Garratty at the American Red Cross Blood Services. Another important class of DIHA-inducing drugs is platinum-based chemotherapeutics, especially oxaliplatin. Historically, alpha-methyldopa has been a major cause.³

Drug reactivity

What causes a relatively small number of drugs to induce antibodies that target red blood cell surface proteins? Any drug can potentially cause DIHA. However, most drugs that have been shown to cause DIHA, often contain at least one chemically reactive group (Table 1).

It is remarkable that among the most commonly implicated drugs (≥ 10 references to DIHA in the literature), nearly all have a clearly identifiable reactive group. Another remarkable finding is that the two most common reactive groups of DIHA-linked drugs are beta-lactam and hydroquinone-like aromatics. Figure 1 shows one example of a drug for each reactive group listed in the table.

Please note that diclofenac itself is not particularly reactive. It does however become reactive when one of our numerous metabolic enzymes, for example cytochrome P450 2C9, attaches an oxygen atom to give the metabolite 4'-hydroxydiclofenac.⁴ Please also note

Table 1 Chemically reactive groups found in 138 drugs or drug-like substances linked with DIHA at least once.³ Two drugs (etodolac and tacrolimus) each contains two such groups, so that the total sum reaches 140 instead of 138. Reactive groups were identified by the authors of this article. The category "Uncertain" does not imply low chemical reactivity, since metabolites may be reactive.

Reactive group	Number of DIHA references in literature (up to 2012)				
	1	< 5	< 10	≥ 10	all
Aldehyde / ketone / acetal	3	4	2	1	10
Aromatic amine / hydroquinone	7	10	1	7	25
Beta-lactam	5	9	5	5	24
Double bond (exo or conjugated)	2	3	2	2	9
Electrophile (non-carbonyl)	3	2	1	0	6
Metal ion	0	1	2	1	4
Thiol	0	1	0	0	1
Thiourea	1	1	0	0	2
Uncertain	29	16	12	2	59
Total	50	47	25	18	140

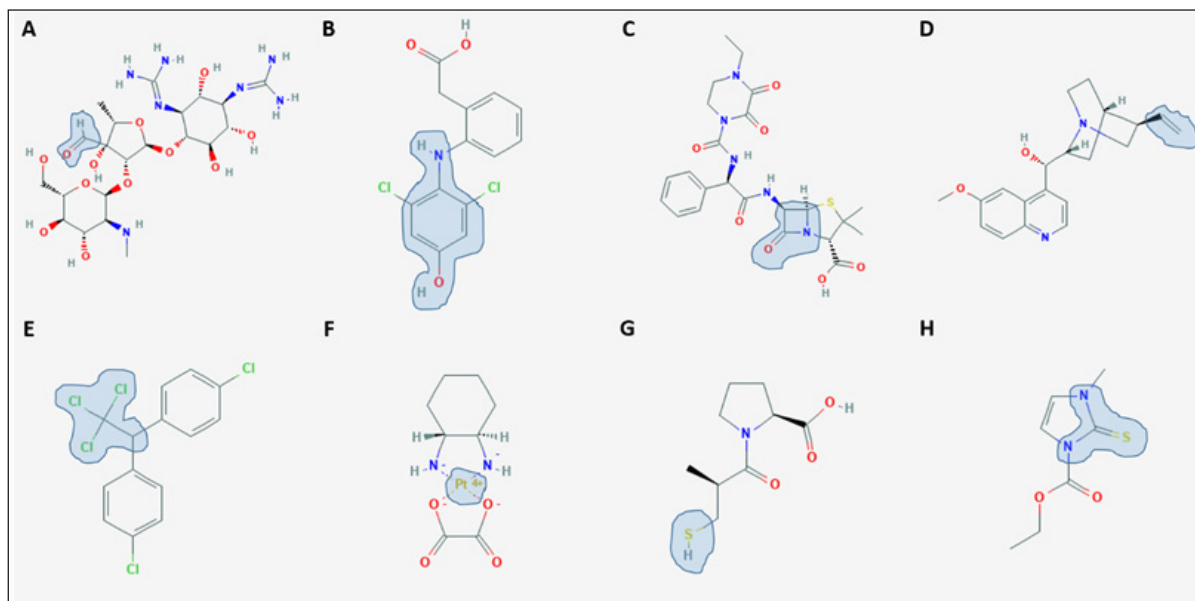


Figure 1 Eight drugs implicated with DIHA multiple times.³ Reactive groups are marked by shaded areas. (A) Streptomycin (aldehyde). (B) Diclofenac metabolite (hydroquinone-like). (C) Piperacillin (beta-lactam). (D) Quinidine (exo double bond). (E) Insecticide DDT (non-carbonyl electrophile). (F) Oxaliplatin (metal ion). (G) Captopril (thiol). (H) Carbimazole (thiourea). Structures were obtained from the PubChem database.⁵

that the insecticide DDT is an example of a reactive “chlorinated hydrocarbon” - Garratty and Arndt do not name it specifically.³

How can we make use of such an overview? We can predict which non-implicated drug may yet cause DIHA. For example, the general anesthetic ketamine contains a reactive ketone group, the cholesterol-lowering drug ezetimibe contains a beta-lactam ring (it is however very lipophilic and may be bound to albumin rather than to the erythrocyte surface), and the “natural” skin-whitening agent arbutin is a hydroquinone which may well cause DIHA if it were administered orally instead of topically.⁵

Most hospitals have a pharmacy or are associated with a pharmacy, and most pharmacists are familiar with the chemical reactivity of drugs. Any pharmacy faculty member is also likely to help when contacted. Please ask if you have a suspicion! It must be said that many drugs, especially the beta-lactam antibiotics, rely on their chemical reactivity for pharmacological efficacy. DIHA is, fortunately, very rare even when reactive drugs such as oxaliplatin are administered daily to thousands of patients worldwide.

Drug-altered red cell proteins

The “hapten” hypothesis suggests that a small, drug-like molecule which is not by itself immunogenic, may become immunogenic when it attaches itself to a protein.⁶ Salama and Mueller-Eckhardt repeatedly remind us that such drug attachment to blood cells is not necessarily very strong, *i.e.* not necessarily covalent.^{1,7} Nevertheless, covalent attachment of drugs to red cell membrane proteins does occur, and Figure 2 shows such a case for the “Band 3” anion exchanger which is one of the most abundant erythrocyte membrane proteins.⁸

The red cell Band 3 protein is an anion exchanger responsible for the Diego, Swann and Froese blood group antigens.¹⁰ There appear to be no cases of DIHA specific for Band 3, unlike for Rhesus proteins, for example.^{2,11} Nevertheless, it is perhaps the first red blood cell membrane protein for which a crystal structure including a covalently attached drug-like molecule has been determined. If we look at the right side of Figure 2, we can imagine how an antibody in the blood might attach more strongly to this new epitope on the Band 3 protein surface, causing its parent B-lymphocyte to

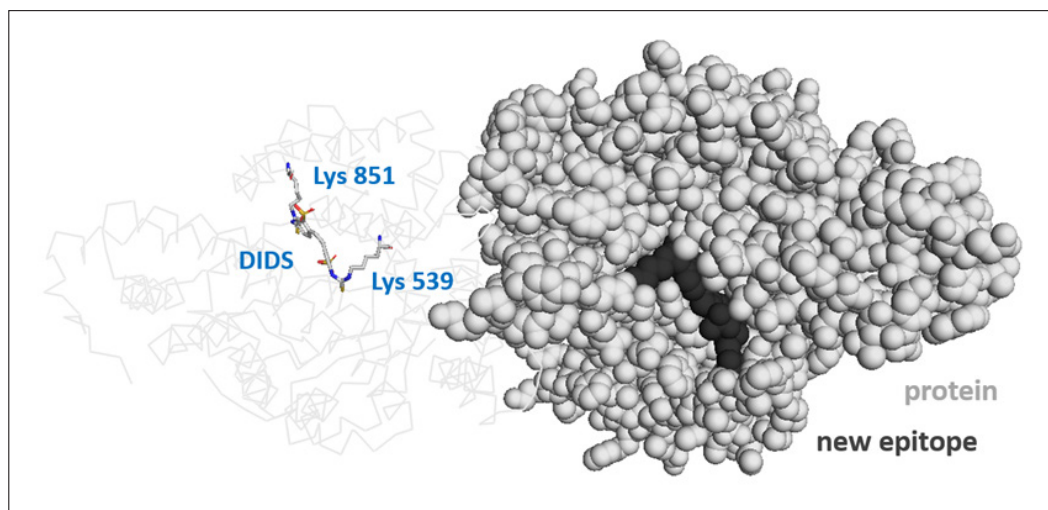


Figure 2 Erythrocyte “Band 3” anion exchanger with the drug-like diisothiocyanostilbene-2,2'-disulfonate (DIDS) covalently bound to two lysine (Lys) sidechains.⁹ This membrane protein forms a homodimer and is shown here as seen perpendicularly from the extracellular (blood plasma) side of the erythrocyte membrane. **Left monomer:** protein backbone ribbon with DIDS and lysines shown in stick representation. **Right monomer:** spacefilling representation, with DIDS and the two attached lysines shown in a darker shade than the rest of the protein. The structure was obtained from the Protein Data Bank¹⁴ (PDB-ID: 4YZF) and the image was generated with PyMOL.

multiply and generate more antibody variants by somatic hypermutation.

Finally, there is one other recent protein crystal structure worth mentioning with regard to DIHA. It is a protein which has attached to it a dopamine metabolite (Figure 3).¹²

Does knowing such structures help us prevent DIHA? Probably not, but at least we may imagine more clearly how drugs and drug-like molecules can alter the surface of red blood cells. Other drug-related immune reactions, such as thrombocytopenia, may occur in a similar way.⁷

Direct Antiglobulin Test (DAT) and DIHA

Does a reasonable suspicion of a DAT test result have to be observed among DIHA patients or donors? A reasonable suspicion of DAT should comprise one part of standard operating procedures, and be performed when physicians or blood bank staff have evidence or cause to suspect a patient or donor of drug use. Evidence is based upon hemolysis, either by trouble shooting the incompatible crossmatches caused by red cells having been coated with IgG, complement, or

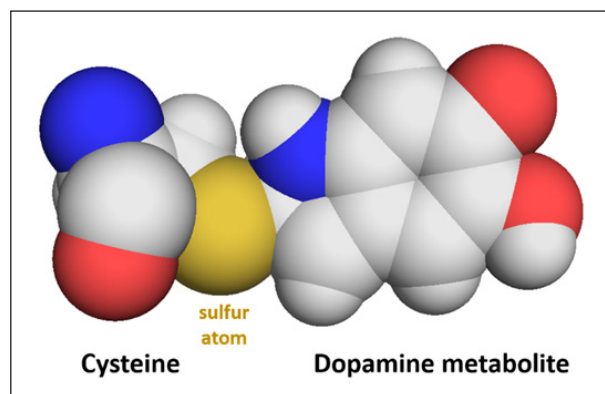


Figure 3 A dopamine metabolite (5,6-dihydroxyindole) covalently attached to the cysteine sidechain of a protein. The drugs alpha-methyl dopa and levodopa, which commonly cause DIHA, are both very similar to dopamine and may undergo a similar attachment to proteins, including red cell surface proteins.¹³ The structure was obtained from the Protein Data Bank (PDB-ID: 6DDA), the image was generated with PyMOL.

both. Several techniques can be used to perform DAT including conventional tube technique (CTT), column agglutination technique (CAT) and flow cytometry. The standard technique is CTT which can detect a level of 100 to 500 molecules of IgG per red cell and 400

to 1,100 molecules of C3d per red cell.¹⁵ Concerning blood donors, positive DAT results without hemolysis have been found in 1:1,000 to 1:14,000 cases depending on various techniques used in different populations,¹⁶ particularly 1:3,000 among Thai blood donors.¹⁷

In the study by Poorekul D and colleagues, 2018, donor samples of 918 positive DAT results were collected from 2005 to 2011 from other hospitals and sent to the National Blood Centre, Thai Red Cross Society. A total of 152 donors (23.2%) reported irregularly positive DAT results which included having a history of medication, infection, allergy, vaccination, taking of supplementary foods and herbs and exposure to chemicals.¹⁹

DIHA requires rare and specialized testing often needed to confirm its diagnosis. Although DAT is a simple test to assist in the diagnosis of DIHA, any additional testing such as the elution technique should be required to provide optimal serological tests for this situation. In these latter cases, the drugs affect the immune system, causing shortened red cell survival with a broad range of severe clinical manifestations.

Conclusion

Some drugs are not only pharmacologically active, but also chemically reactive. They may cause immune hemolytic anemia as well as related immune cytopenias. Drug-induced hemolytic anemia is very rare, but knowing which drugs are more likely to cause it, can help lower the incidence. Regrettably, it's not always easy to correctly diagnose DIHA, a positive direct antiglobulin test is the primary test for proving that an immune event to a distinct drug is occurring. Therefore, the first approach to DIHA therapy is to find out which drug is causing the clinical conditions, and to subsequently stop using the implicated drugs.

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