Immune Complex Formation Involving Etoricoxib in a Patient with Acute Severe Anemia

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Abstract: Background: DIIHA (Drug-induced immune hemolytic anemia) is rare, and a specialized laboratory is often required to provide optimal serological tests to confirm diagnosis. There have been few cases reported of etoricoxib-induced immune hemolytic anemia. Immune complexes formed between some drugs and their respective antibodies attach weakly or strongly in a nonspecific way to RBCs (red blood cells). The bound immune complex activates complement, which may lead to hemolysis *in vivo*. Aims: Demonstration of immune complex formation involving etoricoxib *in vitro*. Methods: A 46-year-old woman developed acute severe anemia one day after a single dose of etoricoxib 90 mg *per os* with a strong positive DAT (direct anti-globulin test) and a weak positive IAT (indirect anti-globuline test). For investigation diagnosis, we used the American Association of Blood Banks Technical Manual protocol using patient serum collected in three different moments (at patient admission, one month after and one month after stopping steroids). Results: The authors found strong positive IAT reactions when the patient serum was tested with the drug. Conclusions: The strong agglutination that occurred in the mixture of the drug and the patient serum indicates a drug/antidrug interaction and may lead to DIIHA. This was the first case reported in Portugal of DIIHA induced by etoricoxib.

Key words: Drug induced immune hemolytic anemia, etoricoxib.

1. Introduction

DIIHA (Drug-induced immune hemolytic anemia) is rare, occurring in approximately 1 in 1 million people [1, 2] but is likely underdiagnosed despite its potential lethality. Nowadays, 2nd and 3rd generation cephalosporins are implicated in the vast majority of cases [2, 3].

A specialized laboratory is often required to provide optimal serological tests to confirm diagnosis. DIIHA antibodies should be classified as drug-dependent (RBC coating; drug-membrane interaction) or drug independent for purposes of diagnosis and treatment [3]. Some reactions are caused by drug metabolites and not the drug itself. Nonetheless, *in vitro* testing may not unravel the true nature of the reaction as the *in vivo* mechanisms of most drug antibody-antigen

interactions are poorly understood and DIIHA is likely mediated by more than one mechanism [2, 4, 5].

In the majority of cases the reaction occurs abruptly, immune complexes formed between some drugs and their respective antibodies may attach, weakly in a nonspecific way, or strongly, to RBCs (red blood cells). The bound immune complex activates complement, which may lead to hemolysis *in vivo*. Different drugs may induce different responses. Usually the DAT (direct antiglobuline test) is positive with anti C3d. Positive anti-IgG is less common, however, during acute phase hemolyses both C3d and anti IgG may be positive and in some cases IgM antibodies might be found. Occasionally drug induced antibodies are indistinguishable from warm IgG antibodies [2, 5].

Etoricoxib, a selective inhibitor of COX-2 (cyclooxygenase 2) is increasingly used in pain relief, namely in osteoarticular pathology. It is mostly used by older women with comorbidities that can cause

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digestive hemorrhage. This can camouflage other causes of anemia misdiagnosing DIIHA.

There have been a few cases reported of etoricoxib-induced immune hemolytic anemia [3].

2. Aims

This study is aimed to demonstrate immune complex formation involving etoricoxib *in vitro*.

3. Clinical Case

A 46-year-old woman was admitted to the ED complaining of increasing asthenia over the past week. She had been medicated with Exxiv® 90 mg the day before for muscular and lumbar pain. On examination, she was pale and had a systolic heart murmur. There was no evidence of fever, active bleeding or drug/toxic exposure. The patient had a history of hypertension treated with amlodipine 20 mg plus olmesartan 5 mg. No other relevant previous clinical history. The

preliminary laboratory results showed an acute severe anemia and the initial immune-hematological study (Table 1) suggested an autoimmune hemolytic anemia. Therefore, anemia etiological study was added, while other causes of anemia were excluded (Table 2) [6]. For life saving purposes, three more ECs (Erythrocyte concentrates) were compatibilized.

The etiological study of acquired hemolytic anemia was negative for autoimmune/aloimmune disease, infection or paraneoplastic syndrome (Tables 1 and 3).

The patient started steroid treatment and transfusion support with progressive increase of hemoglobin levels (Table 1)

One month after the patient referral to ED, both DAT and IAT were negative.

The immune-hematology laboratory decided to continue the etiological study of the hemolytic anemia based on the clinical history and laboratory findings.

Table 1 Laboratory findings.

	Emergency department	1 Month since de beginning of steroid therapy	1 month after withdraw of steroid therapy
Hemoglobin	4.0 g/dL	13.9 g/dL	14.6 g/dL
MCV	118.5 fL	102.7 fL	84.0 fL
MCH	49.4 pg	34.2 pg	29.3 pg
MCHC	41.7 g/dL g/dL	33.3 g/dL	35.3 g/dL
Reticulocytes	12%	2.8%	1.3%
Bilirubin (T)	2.90 mg/dL	0.5 mg/dL	0.6 mg/dL
(Direct)	0.4 mg/dL	--	--
DHL	2,679 UI/L	749 UI/L	535 UI/L
Haptoglobolin	< 6 mg/dL	129 mg/dL	128 mg/dL

Table 2 Etiological study of acquired hemolytic anemia.

Etiological study of acquired hemolytic anemia	
	Normal Protein electrophoresis with Immunoglobulines (A, G, M, D) and complement (C3, C4)—within normal values
Imune (Autoimmune; Aloimmune)	Autoantibodies panel - ANA (Anti- dsDNA + ENA); Anti-cardiolipine IgG/IgM; anti B2 glicoprotein IgM/IgG; Glomerular basement membrane; ANCA (MPO, PR3) ; anti CCP; RP; Lupic anticoagulant - within normal values
Neoplastic/Hipersplenism	CAT scan was unremarkable for neoplastic features or enlarge spleen. Hematological proliferative disease was ruled out upon normal immunophenotyping study.
Infectious	Serology for Epstein Barr, Parvovirus, Toxoplasma Gondii , HCV; HBV; HIV1/HIV2; HTLV I/II—Negative VDRL—Negative CMV DNA—Negative

Table 3 Aetiological study of hereditary anemia.

Etiological study of hereditary anemia	
Hemoglobinopathies	Hemoglobine electrophoresis showed no abnormal bands
Enzimatic defects	No glucose-6-phosphate desidrogenase deficiency
Membrane defects	Blood smear showed no red blood cell fragments and numerous spherocytes. Marked polycromasia. Rare basophile stippling and Howell-Jolly bodies. No sickle cells were observed.

Table 4 Protocol Results.

Results	< 24 h after drug administration		1 Month since de beginning of steroid therapy		1 Month after withdraw of steroid therapy	
	Tube	Card	Tube	Card	Tube	Card
Patient's serum + drug+ rbc's treated with proteolytic enzyme	3+	Neg	2+	1+/Neg	3+	Neg
Patient's serum + drug+ rbc's treated without proteolytic enzyme	3+	2+	Neg	Neg	1+	Neg
Patient's serum + drug+ normal serum + rbc's treated with proteolytic enzyme	1+	1+	1+	1+/Neg	1+/2+	1+/Neg
Patient's serum + drug+ normal serum + rbc's treated without proteolytic enzyme	3+	3+	Neg	Neg	2+	Neg
Patient's serum + normal serum +rbc's treated with proteolytic enzyme	1+	1+	Neg	1+/Neg	Neg	Neg
Patient's serum + normal serum + rbc's treated without proteolytic enzyme	3+	2+	Neg	Neg	Neg	Neg
Drug + normal serum + rbc's treated with proteolytic enzyme	Neg	Neg	Neg	Neg	Neg	Neg
Drug + normal serum + rbc's treated without proteolytic enzyme	Neg	Neg	Neg	Neg	Neg	Neg
PBS + normal serum + rbc's treated with proteolytic enzyme	Neg	Neg	Neg	Neg	Neg	Neg
PBS + normal serum + rbc's treated without proteolytic enzyme	Neg	Neg	Neg	Neg	Neg	Neg

4. Material/Methods

For the immune hematology study, we used the AABBs (American Association of Blood Banks) Technical Manual protocol "Demonstration of Immune complex formation involving drugs." Using patient serum collected in three different moments: firstly we drew blood at patient admission in emergency department (less than 24 h after the patient ingested the drug); the second sample was collected one month after starting steroid therapy and the third sample one month after stopping the treatment with steroids (6 months after the first assay). A drug suspension of 1 mg/mL was prepared using PBS, the pH as adjusted to 7 in order to standardize the reaction. This procedure, using various mixtures of drug suspension and patient serum, enables the demonstration of drug/antidrug interactions using either tube reaction or Diamed-ID micro typing system (Bio-Rad ®). Both tube and micro typing system reaction were graded according to the method specificities described in the literature.

The result interpretation was made according to de AABB orientations. In case of hemolysis or agglutination in reaction containing patient's serum to witch drug was added and the absence of reaction in the corresponding control test, indicates drug/antidrug interaction.

5. Results

At admission, the pre-transfusion immune-hematological study was unable to determine the blood group due to pan-reactiveness. The patient presented a strong positive DAT, with positive reactions for C3d, C3c, IgG, IgM, IgA and card control (the positive control invalidates the tests). We also found a weak positive IAT with pan-reactivity.

We found strong positive reactions when the patient serum was tested with the drug either in the first measurement and the last. The second measurement also showed a positive reaction yet weaker. We also found a weak positive reaction without drug addiction in the first measurement. In all three assays the reaction

was stronger when mixed with normal serum as complement source. All the reactions were stronger when examined for agglutination in tube than when tested in gel ID-card Bio-Rad® (Table 4).

6. Discussion

The authors believe that the strong agglutination that occurred in the mixture of the drug and the patient serum indicates a drug/antidrug interaction. We believe that the reaction found in the first measurement when no drug was added was due to circulating drug in the patient serum, since the drug half-life is of 22 hours. The fact that all the assays showed a stronger agglutination in the presence of normal serum may indicate a complement mediated reaction. The weak positiveness of the third reaction led us to believe the patient serum still had circulating antibodies, however, this hypothesis should have been explored with more exhaustive serological testing. One month after the acute episode the patient presented negative IAT and DAT.

Testing for drug/antidrug interaction is not frequently done in routine laboratories leading to misdiagnosing DIIHA with Idiopathic hemolytic anemia. It is important to achieve definitive diagnosis for it has implications in patient future treatments. Furthermore, more exhaustive protocols would be necessary since the reaction might be secondary to the drug metabolites and not the drug itself. Moreover, drugs such as NSAI (non-steroid anti-inflammatory) may have crossed reaction between different classes.

This investigation should also be conducted in order to protect the patients from future reaction [2, 5].

The DIIAH secondary to the use of NSAI is becoming a pressing topic as the population becomes more aged. The common use of this drug, specially in the elderly, will probably lead to similar reactions in an increased number of patients.

7. Conclusions

In conclusion, once the study pointed to a drug induced hemolytic anemia, the authors decided to report the case to the Portuguese pharmaceutical entities (INFARMED), who considered the drug to be the probable cause for the hemolytic anemia. Moreover, INFARMED confirmed that this was the only case described/reported in Portugal.

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