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SHORT COMMUNICATION

Fatal immune haemolysis due to antibodies to individual metabolites of 5-fluorouracil

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SUMMARY. Confusion still exists in the diagnosis of drug-induced immune haemolysis (DIH). The aim of this study was to demonstrate antibodies specific to 5-fluorouracil (5-FU) in a patient with fatal immune haemolysis (IH). The case of a patient who died due to protracted IH is described. A 57-year-old female underwent treatment with oxaliplatin, 5-FU and folinic acid due to cholangiocarcinoma. Following drug administration, she was transfused because of a mild non-haemolytic anaemia and died following haemolysis. Serological testing including antibody screening, direct antiglobulin test and detection of drug-dependent antibodies was performed using standard techniques. The patient's serum was observed to be red in colour due to the presence of free haemoglobin prior to and following blood transfusion, and contained antibodies reactive with RBCs only in the presence of urine from several patients treated with 5-FU (*ex vivo* antigens). Drug-induced immune haemolysis (DIH) and metabolite-dependent antibodies should always be taken into consideration when a patient being administered any type of drug develops haemolysis.

Key words: drug-dependent antibodies, haemolysis, positive direct antiglobulin test.

Drugs may lead to the production of two types of antibodies: firstly, drug-independent antibodies that react with target cells even in the absence of the drug, demonstrating analogy with true autoantibodies, and/or secondly, drug-dependent antibodies (ddabs) that react with target cells only in the presence of the drug and/or its metabolites (Garratty & Arndt, 2007; Salama, 2008). In rare cases, the metabolite-dependent antibodies are directed against trace and/or individual unknown metabolites, which may be present in the plasma and/or urine of patients receiving the causative drug (Salama & Mueller-Eckhardt, 1985, 1987a,b; Salama *et al*., 1996). 5-Fluorouracil (5-FU) is used as a drug in the treatment of various gastrointestinal cancers (Rochlin *et al*., 1962). To date, there has only been one reported case of 5-FU-induced immune haemolytic anaemia (IHA) in which the causative antibody was observed to react with red blood cells (RBCs) in the presence of the native drug (Sandvei *et al*., 1987). In this study, we describe a patient who developed fatal haemolysis because of a metabolite-dependent antibody that appeared to be directed against individual metabolites of 5-FU

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CASE REPORT

A 57-year-old female who had been suffering from cholangiocarcinoma was administered chemotherapy comprising oxaliplatin, 5-FU and folinic acid on a fortnightly basis for a period of 10 months. There were no signs of haemolysis prior to the last chemotherapy dose, but her haemoglobin concentration was observed to be 8·5 g dL^{-1} (total bilirubin and lactate dehydrogenase levels were normal) (Table 1). Following chemotherapy, she was treated with metamizol (30 drops) for lumbar pain. After 5 h, the patient was administered two units of RBCs. The transfusion was well tolerated and the patient did not complain of any complications. At 6 h following transfusion, the patient was observed to be somnolent. A shock was diagnosed and the following laboratory findings were abnormal: total haemoglobin concentration 9·9 g dL^{-1}, haematocrit 22%, lactate dehydrogenase 4280 U L^{-1}, potassium 6·6 mmol L^{-1} and undetectable

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Table 1. Laboratory findings prior to and following 5-fluorouracil (5-FU) administration

Variable	Range	24 h prior to 5-FU	10 h prior to transfusion and 3 h prior to 5-FU	8 h post-transfusion
Haemoglobin (g dL^{-1})	12·0–15·7	8·4	8·5	9·9[1]
Haematocrit (l L^{-1})	0·35–0·47	0·26	0·26	0·22
Red blood cells (pL^{-1})	3·9–5·4	2·54	2·57	1·79
White blood cells (nL^{-1})	4·5–11·0	3·37	4·56	44·46
Platelets (nL^{-1})	150–400	144	137	196
Sodium (mmol L^{-1})	134–145	137	Not tested	136
Potassium (mmol L^{-1})	3·4–5·2	4·0	Not tested	6·9
Creatinine (mg dL^{-1})	<1·0	1·00	Not tested	2·62
Lactate dehydrogenase (U L^{-1})	<247	171	Not tested	4280
Alanine aminotransferase (U L^{-1})	<34	17	Not tested	43
Aspartate aminotransferase (U L^{-1})	<35	45	Not tested	437
Haptoglobin (mg dL^{-1})	30–200	Not tested	Not tested	<20

[1]Total haemoglobin (free haemoglobin plus that contained within the RBCs).

haptoglobin (Table 1). All therapeutic attempts were unsuccessful and the patient died.

MATERIALS AND METHODS

Oxaliplatin and 5-FU were diluted in 0·9% NaCl at concentrations of 1·0, 0·1 and 0·05 mg mL^{-1}. *Ex vivo* antigens (urine) were obtained from four different patients receiving oxaliplatin, and from six others receiving 5-FU as monotherapy. The direct antiglobulin test (DAT), indirect antiglobulin test (IAT) and test for ddabs were performed as previously described elsewhere, using dialysed (to eliminate drugs and metabolites) and undialysed serum samples and gel cards (DiaMed, Cressier sur Morat, Switzerland) as well as glass tubes (Salama *et al.,* 1992, 1996). Elution of antibodies from RBCs was performed using the acid elution (Immucor, Rödermark, Germany) and heat techniques (10 min at 56 °C).

RESULTS

The patient's serum was found to be red in colour because of haemolysis and the presence of free haemoglobin. The serum was strongly reactive with all tested RBCs using the gel card, and the two transfused units of RBCs appeared to be serologically incompatible due to weak cold antibodies. The DAT was strongly positive with anti-IgG and anti-IgM, weakly positive with anti-C3d and negative with anti-IgA. However, the eluates were non-reactive with RBCs, and serum reactivity with RBCs was abolished following dialysis and elimination of the drugs and metabolites, respectively. In addition, the heat eluate and serum samples demonstrated strong reactions with RBCs in the presence of four of six *ex vivo* antigen samples from patients treated with 5-FU, but not with those from three healthy individuals or patients treated with oxaliplatin (Table 2). No reactions

Table 2. Reactivity of the patient's serum samples with RBCs in the presence and absence of 5-FU and its *ex vivo* metabolites (urine from treated patients)

	Serum dilutions									
	1	2	4	8	16	32	64	128	256	512
Patient's serum plus 5-FU plus RBCs[1]	4+	2+	1+	–	–	–	–	–	–	–
Patient's serum plus saline plus RBCs (negative control)[1]	3+	2+	1+	–	–	–	–	–	–	–
Dialysed serum plus *ex vivo* antigen[2] of 5-FU plus RBCs	4+	4+	4+	4+	4+	4+	3+	2+	+	–
Dialysed serum plus saline plus RBCs (negative control)	–	–	–	–	–	–	–	–	–	–
Heat eluate plus *ex vivo* antigen of 5-FU plus RBCs	4+	4+	4+	4+	3+	3+	1+	–	–	–
Heat eluate plus saline plus RBCs (negative control)	–	–	–	–	–	–	–	–	–	–

[1]Positive reactions are due to the reactivity of ddabs in the presence of the drug and its metabolites. These reactions were abolished following dialysis and drug/metabolite elimination (see negative control and dialysed serum).
[2]Similar reactions were observed with four of six *ex vivo* antigens from patients treated with 5-FU, and no reactions were observed with oxaliplatin and its *ex vivo* antigens.

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were observed with RBCs when *ex vivo* antigen samples were tested with control serum samples obtained from three healthy blood donors.

DISCUSSION

Although drug-induced IH is a well-known phenomenon, confusion still exists in both the bedside and the laboratory diagnosis of the condition. This is reflected in the presented case by numerous aspects. Prior to admission, the patient was repeatedly treated (15 times) with the same combination of drugs without any relevant side effects. On admission, she received her standard chemotherapy dose, and two RBC units were ordered due to mild anaemia. Initially, all serological findings, including the red colour of the serum, positive antibody screening test and positive C3d-DAT, reflected the presence of intravascular IHA. On the contrary, the clinical and laboratory findings prior to chemotherapy did not indicate haemolysis in the patient (Table 1). Consequently, the serological findings were misinterpreted as an *in vitro* phenomenon due to the presence of cold agglutinins.

The cross-match was found to be negative when analysed using the tube technique, 37 °C incubation step and washing procedure prior to the addition of the antiglobulin serum (Coombs serum) to the cell–plasma mixture. This was because the reactivity of the cold and ddab, respectively, was abolished by the incubation at 37 °C and the elimination of the drug during washing. Thus, the transfused RBCs were compatible *in vitro* in the absence of the drug and its metabolite, but not *in vivo* where the drug and its metabolites were still present in the circulation. Consequently, the transfused RBCs appeared to have, at least transitorily, improved the anaemia and the associated symptoms. However, the protracted haemolysis, acidosis and increase in potassium resulted in shock and death. The question as to why the patient did not complain or remained somewhat asymptomatic prior to, during and following blood transfusion remains unclear. The only symptom that appeared to be evidently related to acute haemolysis was the lumbar pain the patient suffered prior to blood transfusion (Garratty & Petz, 2002; Salama, 2008). Although the patient had significant haemoglobinaemia, she did not complain of any symptoms, and the treating physicians were not informed of any unusual events following chemotherapy or blood transfusion. From a clinical point of view, the haemolysis was evidently attributed to the blood transfusion. However, the presence of red coloured serum and the abnormal serological findings indicate that haemolysis was present prior to transfusion. Upon re-questioning and further discussions, it became evident that the haemolysis was related to drug administration. Oxaliplatin was thought to be the primary causative drug. In fact, when the serum was dialysed to eliminate any residual drug and metabolites, its reactivity with RBCs was negated when using the gel card. However, the diagnosis of oxaliplatin-induced IHA could not be verified by serological testing using the drug and its *ex vivo* antigen. In contrast, the dialysed serum sample was observed to react strongly in the presence of four of six urine samples containing the drug and its metabolites. This finding indicated that the causative antibodies were related to 5-FU metabolites that are not invariably produced by all individuals treated with the drug. This finding is supported by previous studies indicating that the causative ddab could be related to trace metabolites from certain individuals (Salama & Mueller-Eckhardt, 1985; Ahrens *et al.,* 2006), and by the well-known interindividual variability in 5-FU metabolism (Niwa *et al.,* 2005).

In our experience, the complexity of the presented case highlights the assumption that the detection of ddabs and the verification of drug-induced IHA are frequently very difficult (Ahrens *et al.,* 2006) or even impossible in isolated cases.

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