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Review paper

Role of red blood cells in pharmacokinetics of chemotherapeutic agents

Dirk Schrijvers, Martin Highley,[1] Ernst De Bruyn,[2] Allan T Van Oosterom[2] and Jan B Vermorken

Department of Medical Oncology, University Hospital Antwerp, Edegem, Belgium. [1]Department of Medical Oncology, Northern Centre for Cancer Treatment, Newcastle General Hospital, Newcastle upon Tyne, UK. [2]Department of Medical Oncology, UZ Gasthuisberg, Leuven, Belgium.

After oral or parenteral administration of chemotherapeutic agents, these drugs are transported to the tissues by the blood in different fractions: plasma water, plasma proteins or cells. In this review, the role of the red blood cell in storage, transport and metabolism of different anti-cancer drugs is described. [© 1999 Lippincott Williams & Wilkins.]

Key words: Chemotherapy, metabolization, red blood cell, storage, transport.

Introduction

The function of the red blood cell has traditionally been considered to be the transport of oxygen by hemoglobin.[1] However, recent findings indicate that red blood cells may also play an important role in the transport of endogenous compounds and drugs, e.g. valproate, phenytoin and hydrocortisone.[2]

After oral or parenteral administration of chemotherapeutic agents used in cancer, these drugs are transported to the tissues by the blood. The free fraction of these drugs acts on tumor cells or the surrounding tissues, e.g. vascular endothelium, and causes tumor necrosis or apoptosis. Transport of these drugs may occur in different fractions: plasma water, plasma proteins and/or cells. The importance of the cellular uptake of cytotoxic drugs has also been recognized recently with respect to tumor kill.

Different methods, each with their own specific problems, have been used to analyze the red blood cell compartment. It is now possible using a specific device to study red blood cells in a reproducible manner, facilitating the study of drugs in this compartment.[2]

In this review, the role of the red blood cell in relation to storage, transport and metabolism of chemotherapeutic agents is described.

Correspondence to D Schrijvers, Department of Medical Oncology, Wilrijkstraat 10, 2650 Edegem, Belgium.
Tel: (+32) 3 821 5306; Fax: (+32) 3 825 1592;
E-mail: Dirk.Schrijvers@uza.uia.ac.be

Anti-metabolites

6-Mercaptopurine (6-MP)

6-MP is an anti-metabolite, used in the treatment of childhood acute lymphoblastic and non-lymphocytic leukemia. After oral administration, 20% of the dose is absorbed and undergoes a first-pass effect in the liver. This prodrug undergoes intracellular metabolism to different nucleotide triphosphates, which, after incorporation into DNA, cause cell cycle arrest and subsequent cell death. In red blood cells, these nucleotide triphosphates are end-stage metabolites, whereas in cells with a nucleus, they are incorporated into DNA. Approximately 20% of the drug is bound to plasma proteins and 10–40% is excreted unchanged in urine. The parent drug has a plasma half-life of 6–10 h.

The concentration of 6-MP and its metabolites in red blood cells can be determined by different methods. Reversed-phase high-performance liquid chromatography (HPLC) has been used to determine extracellular thiopurine nucleotides and bases, methylthiopurine nucleotides and bases, thioxanthine and thiouric acid. An anion-exchange method enables the determination of intracellular mono-, di- and triphosphate (methyl)thiopurine nucleotides. Extraction on ice with perchloric acid and dipotassium hydrogenphosphate results in good recovery of methylthioinosine nucleotides in red blood cells. Measurement of low

concentrations of mono-, di- and triphosphate thioguanine nucleotides in red blood cells (detection limit: 20 pmol/100 cells) is possible after extraction with methanol and methylene chloride, followed by oxidation of thioguanine nucleotides with permanganate and fluorimetric detection.[3]

Another method for the determination of intracellular levels of 6-MP metabolites in red blood cells is capillary electrophoresis with laser-induced fluorescence detection. This method has proved useful in the analysis of intracellular levels of 6-thioguanosine mono-, di- and triphosphate. This assay is linear from 5–1700 pmol/100 ml red blood cells for the three phosphate derivates. This method has been proven feasible for quantification of 6-thioguanine nucleotides in patients receiving either oral or i.v. 6-MP.[4]

Red blood cells may act as a depot for 6-MP metabolites. The rate of accumulation of thioguanine nucleotides and methylmercaptopurine nucleotides has been measured in cell fractions during continuous 6-MP chemotherapy in children with acute lymphoblastic leukemia (ALL). Thioguanine and methylmercaptopurine nucleotide metabolites were present in red blood cells 3 days after oral administration of 6-MP. There was no significant difference between the metabolite concentrations in young, middle-aged or old red blood cells. Therefore, 6-MP metabolites do not specifically enter red blood cells at the stem cell level at the start of therapy but may be taken up by all red blood cells.[5]

The presence of 6-MP metabolites in the red blood cells has important clinical implications. Thioguanine nucleotide concentrations in red blood cells have been correlated with the risk of relapse in 120 children with ALL. Seventeen of 19 relapsing children had significantly lower thioguanine nucleotide concentrations than the group median. Therefore, these metabolites may be an important prognostic indicator in childhood ALL.[6]

Methotrexate (MTX)

MTX is an anti-metabolite, used in the treatment of many solid tumors (choriocarcinoma, breast, head and neck, gastro-intestinal, urological and lung cancers, osteosarcoma, and soft tissue sarcoma) and hematological malignancies (lymphoma, leukemia and multiple myeloma). MTX is usually administered i.v. but after oral administration, 75–95% of the dose is absorbed. Approximately 10% of the drug is metabolized to 7-hydroxymethotrexate and 90% is excreted in the urine unchanged. MTX has a plasma half-life of 3 h.

The concentration of MTX in red blood cells can be determined by HPLC.[7] Flynn *et al.* were able to load erythrocytes with MTX *in vitro* and controlled release was possible by use of a photosensitazation technique.[8]

MTX may accumulate in red blood cells when administered weekly, reaching a steady state after 4–6 weeks. Schroder *et al.* studied 12 children with ALL who were receiving maintenance treatment with MTX and 6-MP. The concentration of MTX in the youngest population of red blood cells contained 2.3–5.9 (mean 3.8) times more MTX than the oldest population. By linear regression analysis, the half-life of MTX in the red blood cells was 19–79 days (mean 37 days). This half-life seemed to be directly related to the amount of MTX which had been metabolized to the 3–5 form of MTX-polyglutamate. The decline of the erythrocyte-linked MTX was predominantly due to selective disappearance of the MTX-glutamate 1 and 2 form, whereas the 3–5 form changed to a much lesser extent with advancing red blood cell age. The steady-state erythrocyte-bound MTX concentration was determined by the amount of MTX added to the circulation by the reticulocytes, the *in vivo* loss predominantly of MTX with low numbers of glutamyl derivatives from erythrocytes and the loss of MTX from destroyed red blood cells. The *in vivo* disappearance of MTX from erythrocytes offers a possible explanation of the observation that the steady-state concentration of MTX in erythrocytes is reached after 4–6 weeks of unaltered weekly MTX treatment.[9]

The intra-erythrocyte storage of MTX was also observed by Lena *et al.*,[7] who determined erythrocyte levels of MTX in patients with solid tumors receiving high-dose MTX treatment. After a first incorporation stage occurring during infusion, MTX concentrations subsequently increased 9–12 days after the treatment as polyglutamized derivatives. Thirty days after the infusion, MTX and its polyglutamates were still measurable in erythrocytes. The percentage of polyglutamization varied on an individual basis, but two groups of patients could be separated according to their ability to form polyglutamates. The presence of 7-hydroxymethotrexate appearing 48 h after the beginning of the infusion could still be detected in the majority of patients (17 of 20 samples) 28 days later.

The presence of 6-MP metabolites in the red blood cells is a prognostic indicator in childhood ALL,[6] but neither the level of erythrocyte-linked MTX nor the folate content of red blood cells is predictive of prognosis. A Pediatric Oncology Group pilot study in 224 children with ALL[10] reported a mean value for red blood cell folate at diagnosis of 0.86 ± 0.46 nmol/ml red blood cells in 214 patients who entered remission,

whilst in 10 patients who failed to achieve remission this value was 1.21 ± 0.74 nmol/ml red blood cells (p=0.02). Folate levels tended to increase during remission induction, but dropped following intensive consolidation with MTX to levels that were sustained throughout chemotherapy. MTX levels reached mean values of approximately 0.15 nmol/ml red blood cells at the end of intensive MTX consolidation, then fell to levels that were sustained throughout maintenance therapy. There was a weak correlation between improved event-free survival and high red blood cell MTX levels after consolidation, but no correlation was found between improved survival and MTX or folate levels during maintenance therapy.

5-Fluorouracil (5-FU)

5-FU is used in the treatment of solid tumors (breast, head and neck, and gastro-intestinal cancer). After i.v. administration, this prodrug is metabolized to different nucleotide triphosphates and acts as a pyrimidine antimetabolite. Less than 10% of the drug is bound to plasma proteins. Approximately 22–45% is metabolized by the liver and 15% is excreted in urine. 5-FU has a plasma half-life of 10–20 min.

An HPLC assay has been used to determine 5-FU in human red blood cells, plasma and whole blood (detection limit: 10 ng/ml).[11]

After i.v. administration of 5-FU, red blood cells contain 55–66% of the plasma concentration and they are responsible for 38.6% of the vascular 5-FU availability. Clearance from red blood cells is increased compared to that from plasma and this may be important in the transfer of this drug from the blood to the tissues.[12] Recent *in vitro* work has shown that 5-FU can induce echynocytosis in erythrocytes, implying higher concentrations of 5-FU in the outer hemileafelet of the erythrocyte membrane.[13] Red cell morphology is rapidly restored to normal when the cells are suspended in a 5-FU-free medium. This suggests that 5-FU is able to freely enter and leave the red cell membrane supporting the concept of the red cell as a transporter of 5-FU.[13]

Enzymes

L-Asparaginase

L-Asparaginase is an enzyme which hydrolyses the amino acid asparagine. It inhibits protein synthesis by depriving tumor cells of asparagine, and is used in the treatment of ALL and non-Hodgkin lymphoma. After intramuscular or i.v. injection, 30% of the drug is plasma protein bound. It has an elimination half-life of 3–5 days. Only traces of L-asparaginase are secreted in urine.

L-Asparaginase is highly toxic, and can cause hypersensitivity reactions and neurological, hematological, gastro-intestinal and hepatic side effects. These problems may be overcome by use of red blood cells.

Kravtzoff *et al.*[14] carried out a study in which 13 patients received a single dose of L-asparaginase (30–200 IU/kg/day). The enzyme was loaded in 1 unit of autologous blood using a lysis-resealing process. A control population of 33 patients receiving L-asparaginase i.v. was tested in parallel. IgG, IgM and IgE class anti-L-asparaginase antibodies were detected using specific radioimmunoassays. L-Asparaginase pharmacokinetic parameters were improved by administration of the drug in red blood cells as compared to i.v. injection, with a prolonged drug elimination. After one injection of red blood cells loaded with L-asparaginase 30 IU/kg body weight, plasma L-asparagine was undetectable after 10 days and after 50 days following 150–200 IU/kg of L-asparaginase. The drug contained in red blood cells was well tolerated and only transient variations in some biological parameters were observed: in one patient a grade 3 thrombocytopenia developed, and eight patients showed moderate and reversible modification of coagulation parameters. No significant clinical toxicity or immune adverse effects were noted. Administration of this drug in red blood cells therefore improves pharmacokinetic parameters and enzymatic efficacy with increased tolerance.[14]

Anthracyclines

Anthracyclines act by intercalating between DNA strands and interfering with DNA replication. In addition, they inhibit topoisomerase II causing DNA double-strand breaks. They are used in the treatment of solid tumors (e.g. breast, lung and ovarian cancers, and sarcoma) and hematological malignancies (leukemia and lymphoma).

Doxorubicin

Doxorubicin is the most commonly used anthracycline. After i.v. administration, 70% is bound to plasma proteins. Doxorubicin is metabolized by the liver to several metabolites, including doxorubicinol. Doxo-

rubicin and doxorubicinol are for 40–50% eliminated by the liver and only 4–5% is found in urine.

Doxorubicin may be detected in plasma, red blood cells, white blood cells and platelets using a fluorimetric procedure or HPLC after *n*-butyl alcohol extraction.[15,16] Colombo *et al.*,[15] who studied the concentration of doxorubicin in a rat model, found that an important fraction of the administered dose was incorporated in red blood cells. After administration of 10 mg/kg, the area under the curve (AUC) of doxorubicin in whole blood was 783 μg/ml.min, in plasma 258 μg/ml.min and in red blood cells 259 μg/ml.min. This indicates that approximately 50% of doxorubicin is transported by red blood cells. When the dose of doxorubicin was increased to 15 mg/kg, the red blood cells accumulated more doxorubicin than plasma, suggesting that the red blood cell compartment has a higher storage capacity.[15]

It is also possible to encapsulate doxorubicin in red blood cells by a dialysis technique with up to 1.6 mg/ml of packed cells. In an *in vitro* model, doxorubicin was slowly released to plasma and efflux of unaltered doxorubicin was observed after 50 h. Intra-erythrocytic metabolism of doxorubicin was restricted to the limited formation of the C-13 hydroxylated metabolite, doxorubicinol.[16] The use of encapsuled doxorubicin with an increased duration of efflux may result in lower cardiotoxicity, since cardiotoxicity is more severe with higher values of the peak concentrations.[17]

Epirubicin

Epirubicin is a less cardiotoxic analog of doxorubicin. It is mainly excreted by the bile and only 10% is eliminated by the kidneys.

Czejka *et al.*[18] studied the disposition of epirubicin and its aglycone in the serum and red blood cells of six patients, after a high-dose i.v. injection, with detection by HPLC. After 20 min, about 50% of the drug was located in red blood cells and 34% in serum. Around 3.5% of the epirubicin-aglycone appeared in the serum within a few minutes and this percentage reached 14% after 4 h. In red blood cells the corresponding percentages were 15% after 10 min and less than 1% after 2 h. When the relative availability in serum and red blood cells were compared, 53% of the epirubicin was accounted for by serum and 50% by red blood cells; with regards to the metabolite, 7% was accounted for by serum and 8% by red blood cells.[18]

The influence of the cytoprotective agent amifostine on the binding of epirubicin to different plasma proteins, control serum, red blood cells and whole blood *in vitro* has been investigated by Pernkopf *et al.*[19] The binding of epirubicin to plasma protein fractions and red blood cells was dependent of the concentration on the matrix components. Epirubicin was bound for more than 90% to human serum α-globulin (α-HSG), 80–90% to human serum albumin (HSA) and human serum β-globulin (β-HSG), and 75% to human serum γ-globulin (γ-HSG). The binding to red blood cells in whole blood samples reached 38%. Amifostine reduced epirubicin binding to all plasma proteins studied: HSA, 2–19%; β-HSG, 4–20%; α-HSG, 2–32%; and γ-HSG, 17–21%. In whole blood samples, the protein-binding of epirubicin decreased from 45 to 32% and red blood cell partitioning from 38 to 32%. This indicates that binding of epirubicin to serum proteins and red blood cells may be reduced in the presence of amifostine.[19]

Bandak *et al.*[20] studied the pharmacokinetic interaction between epirubicin and interferon-α-2b (IFN) in serum and red blood cells in 10 patients. Epirubicin was administered as an i.v. bolus over 2 min at a dose of 60 mg/m^2 after IFN, which was given s.c. 3 times weekly at a dose of 5×10^6 IU. IFN did not significantly influence the pharmacokinetics of epirubicin, e.g. the concentration of epirubicin in red blood cells was reduced from 35.4 to 34.7%.[20]

These results indicate that the concentration of epirubicin associated with red blood cells may be influenced by co-administration of other drugs.

Alkylating agents

Platinum derivatives

Platinum derivatives exert their cytotoxic action by alkylating DNA, thereby interfering with DNA replication. They are used in the treatment of solid tumors (genito-urinary, lung, gastro-intestinal, and head and neck cancers).

Several platinum compounds have been developed. Cisplatin, the original compound, is bound to plasma proteins for 90% after i.v. administration. Between 20 and 45% is excreted unchanged by the kidneys, and it has a half-life of 60–90 h.[21] The derivative carboplatin is active in the same tumor types as cisplatin and is less nephrotoxic and neurotoxic, but more myelosuppressive. Between 60 and 70% is eliminated in urine, and its plasma half-life varies between 2.5 and 6 h.[22] Both drugs may be detected in plasma, and red and white blood cells, by flameless atomic absorption spectrophotometry or by HPLC.[21,22]

Uptake of cisplatin in red blood cells is very rapid although slower than the binding to proteins.[21] The

mean maximum level is lower in red blood cells than in plasma (0.54 versus 1.75 μg/ml). After incubation *in vitro*, the concentrations in red blood cells and supernatant are almost equal, indicating the absence of an active transport mechanism and a low number of binding sites for cisplatin in red blood cells.[21] However, *in vivo* only 1% of the administered dose of cisplatin is located in the red blood cells and it is unlikely that the red blood cells are important in the transport of cisplatin.[23]

Similar observations have been made for carboplatin. Only 0.4% of the dose is transported by red blood cells.[22,24]

Oxaliplatin is a more recently developed platinum complex, active in non-Hodgkin lymphoma, colorectal and ovarian cancers. It is not nephrotoxic or myelotoxic, but neurological disturbances have been observed. After i.v. administration, 66% is bound to plasma. Between 40 and 50% of oxaliplatin is excreted unchanged by the kidneys. It has a plasma half-life of 24 h. The red blood cell concentration after a single i.v. dose is high—at 2 h similar to the plasma concentration—and slowly decreases with a half-life of 230 h, suggesting strong binding to red blood cells.[25]

Protein binding, red blood cell partitioning and biotransformation of oxaliplatin can be studied *in vitro* by atomic absorption spectrometry. The protein binding of oxaliplatin is similar to that of cisplatin and tetraplatin; 85–88% of all platinum from oxaliplatin (5, 10 or 20 μg/ml) is plasma protein-bound within the first 5 h, with an average half-life of 1.71 h. When oxaliplatin is incubated with whole blood (5, 10 or 20 μg/ml), the red blood cell uptake is 37.1% of the total platinum and this is not exchangeable with plasma. The red blood cell fraction does not serve as a reservoir of oxaliplatin.[26]

Red blood cells play only a minor role in the transfer of platinum derivatives to tumor cells.

Ifosfamide

Ifosfamide is an oxazaphosphorine alkylating agent with a broad spectrum of antineoplastic activity (non-seminomatous testicular, head and neck, small and non-small cell lung cancers, pediatric solid tumors, non-Hodgkin and Hodgkin lymphoma, breast, ovarian and cervical cancers, and soft tissue sarcomas). It is a prodrug, metabolized in the liver by cytochrome P450 mixed-function oxidase enzymes to isophosphoramide mustard, the active alkylating compound. Mesna, a uroprotective thiol agent, is routinely administered concomitantly with ifosfamide to prevent ifosfamide-induced hemorrhagic cystitis. The principal dose-limiting toxicity of ifosfamide is myelosuppression. Reversible central nervous system adverse effects ranging from mild somnolence and confusion to severe encephalopathy with coma occur in approximately 10–20% of patients after i.v. infusion. The incidence of neurotoxicity may increase to 50% after oral administration due to differences in the route of metabolism.

Ifosfamide has a half-life of 7–15 h and approximately 15–56% is excreted unchanged in urine.[27]

Oxazaphosphorines and their metabolites may be determined in plasma and red blood cells by gas chromatography–mass spectrometry, and this method has recently been used to study four cancer patients receiving i.v. ifosfamide. A special instrument (MESED-100) was used by Momerency *et al.*[28] to separate a constant volume of red blood cells. Concentrations of ifosfamide, 2- and 3-dechloroethylifosfamide, 4-ketoifosfamide, carboxyifosfamide, isophosphoramide mustard, 2-chloroethylamine, and 1,3-oxazolidin-2-one were determined, and erythrocyte/plasma partition factors calculated. For ifosfamide and metabolites with an intact ring system, partition factors of between 1 and 2 were observed. However, for the compounds with an open structure, carboxyifosfamide and isophosphoramide mustard, partition factors of higher than 3 were obtained. The active anti-tumor metabolite isophosphoramide mustard showed a strong preference for red blood cells. This implies that red blood cells play a role in the transport and subsequent release of the active alkylating agent to tumor cells.[28]

Further data obtained by Highley *et al.*[29] from five patients receiving a 6 h i.v. infusion of ifosfamide showed that the concentration of ifosfamide and its metabolites in the red cell compartment was higher or equal to those in plasma. Isophosphoramide mustard and carboxyifosfamide showed a particular affinity for red blood cells with these cells containing as much as 77% of the total blood concentration of the mustard.

These data show that erythrocyte-associated isophosphoramide mustard is an important transport form of activated ifosfamide.

Taxanes

The taxanes are a new class of antitumor agents with a unique mechanism of action. They promote tubulin polymerization and inhibit depolymerization, causing cell cycle arrest in the G_2/M phase.

Paclitaxel

Paclitaxel was the first drug of this class with activity in ovarian, breast, lung, head and neck, and bladder cancers. It has a α half-life of 0.29 h and a β half-life of 6.95 h, and is mainly excreted unchanged into the bile.

Paclitaxel binds extensively (for about 95%) to plasma proteins including albumin and α_1-acid glycoprotein. Using equilibrium dialysis at clinically relevant concentrations (0.1–6 μM), the binding was found to be independent of concentration, indicating non-specific hydrophobic binding. Human serum albumin and α_1-acid glycoprotein contribute equally to the binding, with a minor contribution from lipoproteins. None of the drugs commonly co-administered with paclitaxel (dexamethasone, diphenhydramine, ranitidine, doxorubicin, 5-FU and cisplatin) alter the binding of paclitaxel to a significant extent. The protein-binding of paclitaxel dramatically decreases the red blood cell uptake, and the red blood cell/buffer ratio falls from 5.81 ± 0.51 to 1.05 ± 0.14 after adding human serum albumin and α_1-acid glycoprotein.[30]

The role of red blood cells in the transport of paclitaxel is thus negligible due to the high protein binding.

Other drugs

Fewer data have been published on other cytotoxic drugs. Camptothecin, a topoisomerase I inhibitor, can exist in two forms; the more active lactone form can undergo hydrolysis to the less active carboxylate form. The lactone form binds to the lipid bilayers of red blood cells 5 times more strongly than the carboxylate form.[31] Erythrocytes therefore act as protectors, stabilizing the lactone ring by preventing its hydrolysis.

Mitomycin C partitions equally between the plasma and the red cell. KW-2149, a recent derivative of mitomycin C, has entered phase I trials but unfortunatelly, lung toxicity has prevented further development. It is known that KW-2148 enters the red blood cell compartment where it may be metabolized to M-18, which is thought to be the active metabolite. Using an *in vitro* model, it has been shown that the concentration of M-18 in erythrocytes can be manipulated with changes in temperature, raising the possibility of M-18 delivery by these cells.[32]

Vinblastine induces microspherocytosis of erythrocytes *in vitro*.[33] This occurs without volume change or external membrane loss, but by the invagination of the erythrocyte membrane through an effect on microfilament proteins. The process can be reversed *in vitro* by removing the vinblastine. Overt evidence of this change is not observed *in vivo* as the concentration of vinblastine required is more than 10 times the usual therapeutic level. There are no data of uptake by erythrocytes *in vivo*. Similarly, etoposide, another well-established cytotoxic agent has not been investigated with respect to pharmacokinetics in red cells.

Conclusion

Red blood cells appear to play an important role in the transport and clinical activity of some anti-cancer agents. Anthracyclines and ifosfamide are for a large part transported by red blood cells, but these cells are not of great importance in the transfer of platinum compounds and taxanes. The incorporation of cytotoxic drugs in red blood cells may ameliorate the toxic effects of these drugs and improve their pharmacokinetic profiles.

Therefore, the role of the red blood cells in the pharmacokinetics of new cytotoxic agents should be assessed in both the preclinical and clinical settings. The incorporation of chemotherapeutic agents in red cells may be of clinical benefit to cancer patients.

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