Review

Defective Drug Uptake Contributing to Multidrug Resistance in Hepatoma Cells Can be Evaluated in Vitro*

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Summary. In clinical practice the acquired or de novo resistance of tumors to antitumor chemotherapy remains a big problem. However, in the past few years some progress has been made in understanding the two principal mechanisms: metabolic alterations leading to a reduced cytostatic or cytotoxic effect of drugs, and reduced accumulation of drugs within the tumor cells [15, 34, 35]. The second phenomenon is usually attributed to the ability of tumor cells to accelerate the efflux of various xenobiotics. This phenomenon is considered primarily responsible for the development of multidrug resistance (MDR). However, loss or impairment of drug uptake by the tumor cells may also contribute to resistance to antitumor drugs. This paper focusses on recent findings with hepatoma cells, which support this view.

Key words: Tumor cell resistance – Hepatoma – Bile salts – Fluorescence microscopy

Mechanisms of Tumor Cell Resistance with Special Regard to Hepatoma Cells

Some malignant tumors, for instance, most colorectal and hepatocellular carcinomas, are inherently resistant to chemotherapy; others develop resistance only after a drug is administered, and show – when the therapeutic regimen is altered – resistance to others as well (cross resistance).

Metabolic Detoxication. In some tumors, especially those which were primarily resistant to antitumor chemotherapy, uncommonly high concentrations of glutathione were found. In hepatocytes, the increasing resistance to the toxic effect of aflatoxin B1 was correlated with increasing intracellular concentrations of glutathione and increasing activity of glutathione-S-transferase [33, 41], enhancing the metabolic detoxication of aflatoxin B1. These considerations suggested that the primary resistance of colorectal tumors to chemotherapy may be related to the extraordinary high content of GSH in the colorectal mucosa. It was assumed that enhanced conjugation of the drugs with glutathione was decisive in the reduction of their chemotherapeutic effect. However, in resistant tumors, the increased activity of the glutathione-S-transferases could be attributed mainly to one of its isoenzymes, which possesses a peroxidative function [4]. This led to the hypothesis that the cytotoxic effect of adriamycin and similar drugs was due to generation of oxygen radicals, and that detoxication of these radicals by the enhanced peroxidating activity of the specific GSH-transferase accounted for the increased tumor cell resistance [27].

Carrier-Mediated Extrusion of Chemotherapeutic Agents. The development of resistance of leukemia cells and Ehrlich ascites tumor cells to daunorubicine and vincristine corresponds to an enhanced elimination of these drugs from the cells by the induction of an active, carrier-mediated extrusion process [12, 23, 24, 43]. With repeated exposure, normal cells also develop an increasing resistance to carcinogens. Moreover, the cells not only become resistant to the resistance-inducing agent but also to others such as methotrexate, adriamycin, cycloheximide, and aflatoxin B1 [9, 33, 40]. The inducible extrusion process is apparently related to a 170 kDa glycoprotein in the plasma membrane, called P170, which is thought to be involved normally in the elimination of xenobiotics [11]. Its
Loss of Uptake Functions. It has only recently been appreciated that impairment of the uptake of xenobiotics across the plasma membranes of tumor cells could be a factor in tumor cell resistance [22]. Only a few deficiencies in specific transport functions have been found in some transformed cell lines, e.g., the hypoxanthine transport deficiency of S49 mouse lymphoma cells [39] or the transport deficiency of purine and pyrimidine nucleosides in mouse lymphoma cells [10]. In hepatoma cells, glucagon receptor and postreceptor defects were found that caused glucagon resistance [14]. None of the above defects, however, were correlated with a deficiency in uptake of antitumor drugs. By contrast, the deficiency in uptake of 5-methyltetrahydrofolate by hepatoma cells also impairs the uptake of the folate derivative, methotrexate [19]. Recently, other deficiencies in other uptake functions, e.g., for bile salts [8, 29, 48, 51], taurine [31], cysteinyl leukotrienes [49], and bilirubin [44] have been detected in hepatoma cell lines.

The loss of the ability to take up bile salts [8, 29, 48, 51] is of particular interest since the uptake system(s) responsible exhibit(s) broad substrate specificity and is/are also responsible for the transport of a variety of xenobiotics [2, 6, 7, 15, 18, 36, 37, 38, 50, 51]. Thus, loss of bile salt uptake might also mean loss or deterioration of the uptake of some drugs such as ajmaline [7] or steroid hormones [6, 7]. By photoaffinity labelling using photolabile bile salt derivatives [6, 27, 30, 48, 51], the membrane polypeptides with apparent Mr's of 46000 [30], 48000 [30], and 49000 [1, 32], which were suggested to be involved in the hepatic uptake of bile salts in normal hepatocytes, could not be detected in various hepatoma cell lines [8, 48]. Furthermore, monoclonal antibodies against the 49 kDa polypeptide, which bind to the surface of normal hepatocytes, failed to bind to hepatoma cells [32]. Thus, the loss of bile salt uptake by hepatoma cells apparently correlates with the loss or a structural alteration of bile salt-binding membrane polypeptides, and has been shown to impair uptake of some antitumor drugs that share this transport system, e.g., methotrexate [18, 21]. Moreover, 5-fluoro-2-deoxyuridine (which by linkage to a bile salt was designed to be taken up by the bile salt carrier(s) and therefore to be liver specific [16]) can be expected to be taken up by normal hepatocytes but not by hepatoma cells lacking the bile salt uptake system(s).

Clinical Relevance

The knowledge of the drug response of tumor cells is of major importance in planning therapeutic regimens. Drugs which are rapidly detoxified, extruded, or even excluded by tumor cells exhibit no therapeutic benefits and only engender side effects. Usually, drug resistance is detected by studying the effect of special antitumor drugs on viability and growth of isolated, cultivated, or transplanted tumor cells, which is a complicated and prolonged procedure. However, if drug resistance is due to loss or deterioration of its uptake, more simple and rapid testing may be possible.

The uptake of antitumor drugs can be directly observed by microscopy, if they are fluorescent, e.g., adriamycin, daunomycin, and daunorubicin [3, 13]. Although most other chemotherapeutic agents are not inherently fluorescent, fluorescent derivatives can be prepared that share the same transport systems. Thus, a fluorescent derivative of methotrexate is now on the market to test resistance of tumor cells to this drug [26, 42]. By the use of the fluorescent bile salt derivative, (N-[7-(4-nitrobenzo-2-oxa-1,3-diazol)]-7β-amino-3α,12α-dihydroxy-5β-cholan-24-oyl)-2-aminoethanesulfonate, one can easily investigate the capability of transformed hepatocytes to take up special xenobiotics [5, 7] the uptake of which depends on functioning bile salt uptake. While it is taken up by normal hepatocytes, it may be excluded by hepatoma cells [8] indicating that the uptake of all drugs including chemotherapeutic agents, e.g., methotrexate [18, 21], the uptake of which depends on (a) functioning bile salt uptake system(s) may also be deteriorated or completely lacking. It is likely that fluorescence microscopic studies on the uptake properties of isolated, cultivated, or transplanted tumor cells using fluorescent compounds will be extremely useful for the design of chemotherapeutic regimens. Therefore, it seems worth-