In our recently published cohort study (1), we observed the presence of atrophic gastritis in 62% of 24 cagA-positive patients, compared with 32% of 34 cagA-negative patients, corresponding with an odds ratio (OR) of 3.48 (95% confidence interval [CI] = 1.02-12.2). In a previous, prospective, nested case-control study (2), we observed the development of gastric cancer in 109 subjects who had had a phlebotomy 13 ± 5 years (mean ± standard deviation) earlier. On the basis of serologic testing, 103 (94%) of these subjects had been infected with H. pylori at the time of bleeding. In an extension of this study, it appeared that 90 (87%) of these 103 patients with gastric cancer had been infected with cagA-positive strains (3). This proportion was 78% in 103 matched H. pylori-infected control subjects who did not develop gastric cancer during follow-up, corresponding with an OR of 1.9 (95% CI = 0.9-4.0) (3). The association between infection with a cagA-positive H. pylori strain and gastric cancer was strongest for intestinal-type carcinomas of the distal stomach (OR = 2.3; 95% CI = 1.0-5.2) (3). Ponzetto et al. found cagA antibodies in 96% (95% CI = 87-100) of 51 patients with gastric cancer. This percentage is in agreement with our previous findings, but is remarkably high considering that the specimens were from patients who already were diagnosed as having cancer. During the process of the development of atrophy and cancer, signs of a pre-existing H. pylori infection may disappear (4). The 18% prevalence of cagA antibodies in the control subjects, on the other hand, seems rather low when compared with other studies. It is not clear whether or not all of the 555 consecutive individuals tested were H. pylori positive.

In total, the findings of Ponzetto et al. are very provocative. They would be best served by a fuller exposition of the data, including formal information on the accuracy of the diagnostic tools used, rates of infection with cagA-positive strains in appropriate controls, location and histologic type of the tumors, and H. pylori status of patients and controls. We look forward to this more extensive publication.

References


Note

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Re: How Does the MRP/GS-X Pump Export Doxorubicin?

A recent correspondence to the Journal (1) addressed the role of the MRP gene-encoded multidrug resistance protein (MRP) (2) in cytostatic drug resistance. It has been shown that transfection of the MRP gene induces transport of the cytostatic drug doxorubicin (3) as well as adenosine triphosphate (ATP)-dependent transport of glutathione S-conjugates (GS-X) (4), the organic anion calcein (5), and the heavy metal ion arsenite (6). The discussion in the above-cited correspondence addressed the question of how one protein might mediate the active transport of anions (dinonitrophenyl-S-glutathione and calcein) as well as neutral (etoposide) and cationic (doxorubicin, daunorubicin, vincristine, and rhodamine 123) lipophilic drugs.

In our contribution to this discussion, we wish to summarize some related evidence that has not been adequately considered. Ishikawa et al. (1) tried to find explanations for the fact that doxorubicin efflux from cells is not remarkably enhanced by transfection with the MRP gene alone. Their hypothesis is that doxorubicin must first be conjugated to glutathione in a series of chemical reactions in order to be recognized as a substrate for MRP.

To our knowledge, convincing data excluding the possibility that nonconjugated doxorubicin is actively transported from MRP gene-transfected cells have not been published. Demonstration of such doxorubicin transport requires a sensitive, carefully controlled experimental setup because of extensive binding of this drug to plastics and proteins. In addition, the ratio of active to passive transport that determines the accumulation deficit caused by P-glycoprotein or MRP is not as favorable for doxorubicin as it is for other substrates (e.g., rhodamine 123 or calcine-AM for P-glycoprotein). Therefore, since available MRP gene-transfected cells do not exhibit very high MRP overexpression, active doxorubicin transport might easily be missed. The apparent absence of ATP-dependent transport of doxorubicin in MRP overexpressing inside-out vesicles (1) is not definitive evidence of the absence of such transport.

On the other hand, some data on MRP-mediated transport are consistent with the transport of unmodified drug molecules. First, a much more rapid efflux rate has been shown for daunorubicin and etoposide with MRP-overexpressing cells than with drug-sensitive cells (7). This efflux rate is comparable to efflux rates in cells highly overexpressing Pgp (8). However, it has not been found that glutathione conjugates of daunorubicin are formed in tumor cells in amounts appreciable enough to account for this rapid efflux (6). In addition, if labile glutathione conjugates were pumped into the culture medium by MRP, then an increase in glutathione efflux should have been found at maximal pump rates for daunorubicin. This was not the case (9).
However, during the transport of arsenite from MRP-overexpressing cells, an increase in glutathione efflux into the culture medium has been observed (6). Thus, MRP may very well pump glutathione complexes of arsenite, which are formed easily. Furthermore, any explanation in favor of exclusive transport by MRP of negatively charged drug conjugates has to deal not only with anhydraclycines, but also with vincristine, etoposide, and rhodamine 123 transport. The need for extensive conjugation in tumor cells of such a variety of compounds makes this hypothesis even more unlikely.

We think that, in order to explain the available data, it is not necessary to postulate the formation and subsequent transport by MRP of glutathione–doxorubicin, glutathione–etoposide, or glutathione–vincristine conjugates. Nevertheless, it is clear that partial glutathione depletion of tumor cells inhibits MRP, but not P-glycoprotein–mediated transport of these drugs, without having an effect on the pump rate of the organic anion calcine (10). Therefore, we advance the hypothesis that glutathione is needed to keep MRP in a conformational state that allows the transport of neutral or positively charged molecules, perhaps as part of a ternary complex.

It is not known whether the putative human liver-specific MRP homologue or canalicular multispecific organic anion transporter (cMOAT), which pumps a broad range of multivalent organic anions into bile, also pumps cytostatic drugs. The recent cloning from rat hepatocyte DNA of an rMRP2 gene, different from the rat or human MRP and absent in rats with a deficient cMOAT (TR–), suggests that it may be the rat cMOAT gene (11). Elucidation of the transport mechanism of MRP and its liver-specific MRP-homologue will require further research, such as purification and functional reconstitution of MRP and cMOAT and detailed analyses of transport kinetics.

With respect to cancer chemotherapy, it is of interest that the early data suggest that MRP is expressed differently in several tumor types (12) and that it is present mainly in cellular plasma membranes in MRP-positive tumors (13). Therefore, the concept of increasing the cellular uptake of MRP substrates in MRP-positive tumors can now be considered in the clinic. In particular, combinations of etoposide and anthracyclines with MRP inhibitors, inhibitors of organic anion transport such as probenecid, benzamorfone, or the leukotriene receptor antagonist MK571, or in combination with partial glutathione depletion with buthionine sulfoximine (10), can be envisaged as a workable approach. Whether such an approach will lead to improved response to chemotherapy in certain patients may depend on largely unknown factors related to the in vivo role of MRP, just as has been argued for P-glycoprotein (14).

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Response
We thank Broxterman et al. for their interest and critical comments regarding our recent correspondence to the Journal (1). In our letter, we proposed a putative metabolic pathway to explain how the MRP/GS-X pump (2) might export doxorubicin. Our hypothesis was based on recent findings that: 1) unmodified doxorubicin is a very poor substrate for the GS-X pump using a membrane vesicle transport assay system (Ishikawa T, unpublished results); 2) adenosine triphosphate-dependent transport by the MRP/GS-X pump of GS-conjugates and leukothione C4 in plasma membrane vesicles prepared from MRP-complementary DNA-transfected cells is reportedly not inhibited by doxorubicin (3); and 3) the 190-kd MRP/GS-X pump protein in MRP-overexpressing H69AR cells is not labeled with a photoaffinity analogue of doxorubicin (4). Since several recently published results suggest that MRP may function as a GS-X pump mediating the transport of glutathione S-conjugates, such as cysteiny1 leukotrienes (3,5) and glutathione–heavy
metal complexes (6-8), we therefore considered the possibility of glutathione conjugation as a potentially important mechanism for the transport of doxorubicin in MRP-overexpressing cell lines. Carefully considering the biochemistry of anthracyclines, we proposed that intracellular doxorubicin might first be reduced to form an active aglycone intermediate (perhaps via a reductase such as DT-diaphorase), which then reacts with glutathione (GSH) to form GS-doxorubicin that may be recognized and transported by the MRP/GS-X pump (7). Such bioactivation and thiol-conjugation of anthracyclines (9,10) have unfortunately not been considered by many researchers.

The molecular basis of drug resistance in MRP-overexpressing cell lines is likely to be very complex (11). A very critical issue here is the lack of a clear understanding at the present time regarding the substrate specificity of the MRP/GS-X pump. Although there are no convincing data excluding the possibility that doxorubicin is not a substrate for the MRP/GS-X pump, as mentioned by Broxterman et al., there is no convincing evidence published in the literature to confirm that unmodified doxorubicin can be handled by this efflux pump. Furthermore, no convincing kinetic data (e.g., $K_m$ and $V_{max}$) have been reported for the transport of doxorubicin mediated by MRP. The putative metabolic scheme that we proposed (7), taking into consideration the possible complex molecular mechanisms of MRP-mediated drug resistance described in the literature, suggests that not only the MRP/GS-X pump per se, but also other cellular factors, including intracellular GSH metabolism and DT-diaphorase activity, may be of critical importance in contributing to the drug-resistance observed in MRP-overexpressing cells. Most important, our scheme provides a testable approach to address whether glutathione conjugation of doxorubicin is an important step for MRP-mediated drug resistance. Experiments addressing these relevant issues are currently under way in our laboratories.

Note added in proof: After submission of our reply, Jedlitschky et al. (12) reported that the MRP/GS-X pump transports glutathione, glucuronate, and sulfate conjugates but not unmodified doxorubicin, daunorubicin, or vinblastine, in support of our idea (1,11). Furthermore, we have now provided evidence for coordinated induction of both the MRP/GS-X pump and $\gamma$-glutamylcysteine synthetase, which strongly suggests that the physiologic function of the MRP/GS-X pump is closely linked with cellular glutathione biosynthesis (13).

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