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Liver Targeting of Autologous Erythrocytes Loaded with Doxorubicin

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ERYTHROCYTES HAVE been proposed in animal models as carriers of cytotoxic drugs or as bioreactors converting prodrugs to active drugs [1, 2]. Doxorubicin has been encapsulated in human and animal erythrocytes [3–7]. Treatment of the doxorubicin-loaded erythrocytes with glutaraldehyde, a bifunctional reagent, prevented the fast efflux of doxorubicin from the cells and produced their specific targeting to the liver of mice and dogs [4–7]. The present research aimed to extend these experimental studies to the clinic.

A 51-year-old male had a colorectal carcinoma, massively metastatic to the liver (60% tumour involvement). The primary tumour was removed surgically and, at the time our study started, the disease was confined to the liver and slowly progressing. The patient had an ECOG performance status of 2. He had previously failed three lines of chemotherapy (5-fluorouracil, fotemustine, mitomycin), either systemic or locoregional, through a Port-a-cath implanted in the hepatic artery at the time of surgery. Informed consent was obtained from the patient.

Freshly drawn erythrocytes were loaded with doxorubicin by simple diffusion [6]; the yield of encapsulation of the drug was 2 mg/ml of packed cells. Glutaraldehyde treatment (0.15% and 0.30% final concentration) was performed after encapsulation of doxorubicin [6]. This treatment substantially reduced the rate of doxorubicin efflux *in vitro* (more than 70% of the drug retained after 210 min at 37°C). For *in vivo* distribution studies erythrocytes were labeled with ^{99m}Tc [8] prior to encapsulation. All manipulations were performed under sterile pyrogen-free conditions.

Figure 1 shows the time-radioactivity curves over the regions of liver and heart, after administration through hepatic artery of ^{99m}Tc labeled native (a) and unloaded GA treated (b) erythrocytes. Unlike the native cells, showing a rapid transit from the liver to systemic circulation (a), the glutaraldehyde treated erythrocytes were almost completely retained by the liver (b), up to 24 hours after injection (not shown). No accumulation of

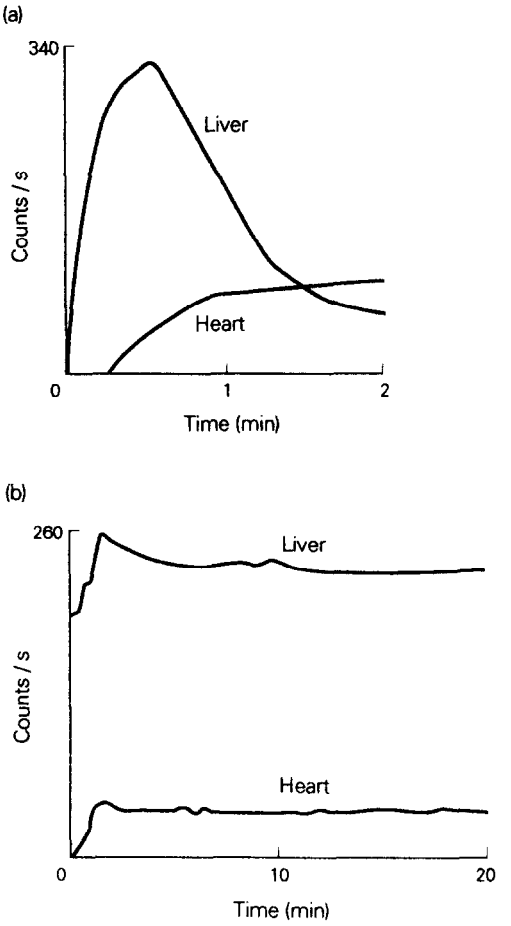

Fig. 1. Variations in radioactivity over the region of liver and heart as measured by computerised analysis during the dynamic studies. 2 ml Tc^{99m} labeled native erythrocytes (a) or Tc^{99m} labeled glutaraldehyde-treated erythrocytes (b) were injected into the hepatic artery as bolus.

radioactivity was observed in other organs. Doxorubicin-loaded, glutaraldehyde-treated erythrocytes showed a time–activity profile identical to unloaded glutaraldehyde-treated erythrocytes. Radioactivity was also monitored for 1 hour in the blood: a few minutes following administration of native erythrocytes, 90% of injected radioactivity was found in the general circulation, whereas 10% only was observed with the glutaraldehyde-treated cells.

The systemic plasma pharmacokinetics of doxorubicin and its metabolites was also investigated [7]. The drug (10 mg/m^2) was administered at weekly intervals, either free (3 times) or encapsulated in glutaraldehyde-treated erythrocytes (6 times), through the hepatic artery (infusion rate 1 mg/min; total dose: 150 mg/m^2; dose intensity: 5 mg/m^2/week). Doxorubicin-loaded and glutaraldehyde-treated erythrocytes resulted in a consistent reduction of peak plasma levels (140 mg/ml) and of area under the curve (AUC) values (130 μg.h/l) of the parent drug, compared with administration of the same dose of free doxorubicin (485 mg/ml and 228 μg.h/l, respectively). These data indicate an increased hepatic extraction of the erythrocyte-encapsulated drug, lower systemic exposure and possibly a higher local concentration.

The results obtained indicated that administration of doxorubicin-loaded and glutaraldehyde-treated autologous erythrocytes resulted in extensive accumulation of both carrier cells and drug in the liver.

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Neuron-specific Enolase (NSE) in Bronchial Washings: a Better Diagnostic Marker for Small Cell Lung Cancer

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SMALL CELL lung carcinoma (SCLC), which accounts for about 20% of lung cancers, is the most sensitive to chemotherapy and radiation. This has emphasised the importance of an accurate diagnosis of this cell type. Enolases are glycolytic enzymes catalysing the conversion of 2-phosphoglycerate to phosphoenolpyruvate. There are three dimeric isoenzymes, one of which is found in neurones and cells of neural origin; this has been called neuron-specific enolase (NSE) [1, 2].

SCLC shows neuroendocrine properties and a positive correlation between serum NSE and extent of disease has been shown [2–4]. Serum NSE concentration was raised (>12.5 ng/ml) in 60–77.5% of patients with SCLC. The 80–91% of patients with extensive disease and the 37–50% of patients with limited disease were serum NSE positive [2–7].

The diagnostic significance of NSE, in both serum (S-NSE) and bronchial washings (BW-NSE) aspirated during fiberoptic bronchoscopy after addition of 10 ml normal saline was examined in 14 patients with SCLC (6 of them with extensive disease and 8 of them with limited disease), as well in 20 patients with extensive inoperable non-small cell lung cancer (NSCLC). We examined also a group of 9 individuals who underwent fiberoptic bronchoscopy for other reasons (persistent cough, undiagnosed pleural effusion, haemoptysis with normal chest X-ray) in order to obtain the normal concentration of NSE in bronchial washings. The results of the control group showed that the range of BW-NSE values in non-malignant lung disease was 2.2–13.7 ng/ml (mean [S.D.] = 8.3 [6.2] ng/ml) and the S-NSE was within normal values (<12.5 ng/ml) in all of them.

All the patients with extensive SCLC showed a rise in S-NSE (23 [5] ng/ml) and all presented extremely raised levels of BW-NSE (79.3 [26.4] ng/ml).

The 25% of patients with LTD-SCLC had increased levels of S-NSE (19.3 [6.3] ng/ml), while the other 75% showed a significant rise of BS-NSE (49.8 [17] ng/ml).

The 35% of patients with NSCLC presented a moderate rise of S-NSE (16.8 [8.3] ng/ml) and BW-NSE (20.6 [12.2] ng/ml), while the rest showed levels of S-NSE and BW-NSE similar to those of the control group.

These results are preliminary, so we are not yet able to support them by a reliable quantitative statistical analysis. It has become obvious using the initial results that the sensitivity of BW-NSE in SCLC (85.7%), especially in limited disease, was greater then the sensitivity of S-NSE (57%), while the specificity of both was equal (76%).

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