Evaluating Bone Marrow Metastasis of Neuroblastoma with Iodine-123-MIBG Scintigraphy and MRI

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Of 10 patients with neuroblastoma who had both ^{123}I-MIBG scintigraphy and MRI at diagnosis, four presented with bone marrow metastasis that was diagnosed by both imaging modalities and confirmed by bone marrow biopsy and smears. This report focuses on the follow up of the four patients with bone marrow metastasis. MIBG scintigraphy and MRI were concordant in two patients, a case of normalization and a case of relapse in the seventh dorsal vertebra confirmed by surgical biopsy. The last two patients presented a normalized MIBG scan for marrow infiltration after chemotherapy but persistent abnormal MRI signal of several vertebrae, suggesting marrow infiltration, up to 27 mo after the end of chemotherapy in one case. In the second patient, MRI bone marrow aspect returned to normal 4 mo after the end of chemotherapy. Bone marrow biopsy remained negative in these two MIBG-negative patients. These cases suggest that in presence of complete normalization of the MIBG scan after chemotherapy, the persistence of a hypointense signal on bone marrow on T1WI does not necessarily indicate persistence of disease but may be due to delayed normalization. Therefore, attention must be paid to the delay of signal normalization on MRI (which can be as long as more than 2 yr after the end of chemotherapy) in order to avoid false-positive interpretation.

Keys Words: metaiodobenzylguanidine; magnetic resonance imaging; bone marrow; neuroblastoma

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Bone marrow metastasis of neuroblastoma is conventionally detected by cytological and histological examination of marrow issued of both posterior iliac crest (*1*). However, the area examined is limited and neuroblastoma is a focal disease. For this reason sensitive techniques exploring larger territories of bone marrow are important, especially in the follow up, to allow a more accurate evaluation of response to treatment. The usefulness of MIBG scintigraphy has been widely demonstrated in the diagnosis, staging and follow-up of neuroblastoma (*2–10*). The great sensitivity of MRI in detecting bone marrow abnormalities has been described (*11–12*). This report focuses on the role of both imaging modalities in the evaluation of the response to treatment in four patients with histologically-proven neuroblastoma who presented with bone marrow metastasis at diagnosis. The chemotherapeutic regimen adopted was the same for all patients (vincristine, cyclophosphamide and adriamycine, in alternance with VP16 and cisplatine). MIBG scans and MRI were performed before, during and after chemotherapy (follow-up period). Bone marrow was aspirated and biopsies of the bilateral posterior iliac crest were performed for histological, cytological and immunohistochemical assessments.

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CASE REPORTS

Patient 1

A 3-yr-old boy was admitted to the hospital because of behavioral disorder, drowsiness and vomiting occurring predominantly in the morning. Different radiological procedures (US, CT scan, MRI) showed a right adrenal mass associated with a large tumor of the base of the skull. Moreover, MRI showed a diffuse hypointense signal of the vertebral bodies which suggested bone marrow infiltration. MIBG scan showed a high uptake in the right adrenal mass and in the tumor of the base of the skull and also abnormal uptake in the spine, pelvis and femurs that reflects massive bone marrow infiltration. Urinary catecholamines were elevated. Bone marrow biopsy revealed marrow infiltration by tumoral (neuroblastoma) cells. Histology confirmed the diagnosis of right adrenal neuroblastoma with metastasis to the base of the skull. In the evaluation of the response to treament of bone marrow infiltration, both MIBG scan and MRI were concordant and became negative which suggest complete healing of marrow disease after therapy, confirmed by bone marrow examinations which also became negative; 1.5 yr after diagnosis, the patient relapsed in the primary metastasis site (base of the skull) with subsequent dissemination and died from disease.

Patient 2

A 6-yr-old boy was admitted to the hospital for diarrhea with griping pain; these episodes occurred as many as 15 times per day. Chest radiography, US, CT and MRI were performed and showed a left paravertebral mass extending from T7 to L1 that infiltrated the right paravertebral groove and the intervertebral foramen of T10 and T11. Also, the MRI showed a pathological signal of T7 and L3. The MIBG scan, performed postoperatively, showed a pathological uptake at the level of T7 and L3 and an additionnal site of abnormal uptake in the lumbar spine (most likely L5). These abnormal foci suggested bone and/or bone marrow metastases. Urinary catecholamines were elevated. Bone marrow biopsy revealed clumps of neuroblastoma cells. Histology of the primary paravertebral tumor diagnosed a ganglioneuroblastoma with lymph nodes metastases. Fourteen months after the end of treatment, the patient presented abnormal MIBG uptake and MRI signal in the same dorsal vertebra involved at diagnosis (T7). Bone marrow biopsy from the iliac crest was negative. A subsequent surgical bone biopsy of the seventh dorsal vertebra, however, showed a bone metastasis, and this patient developed diffuse bone marrow involvement 2 mo later. He died from disease 20 mo later.

Patient 3

A 5-yr-old boy was admitted to the hospital because of intermittent pain of the lower legs associated with left cervical adenopathy, weight loss and tiredness. MRI showed a right adrenal mass, bilateral paravertebral metastatic masses and diffuse hypointense signal of the vertebral bodies compared to the humeral epiphysis and diaphysis suggestive of an almost complete infiltration of the

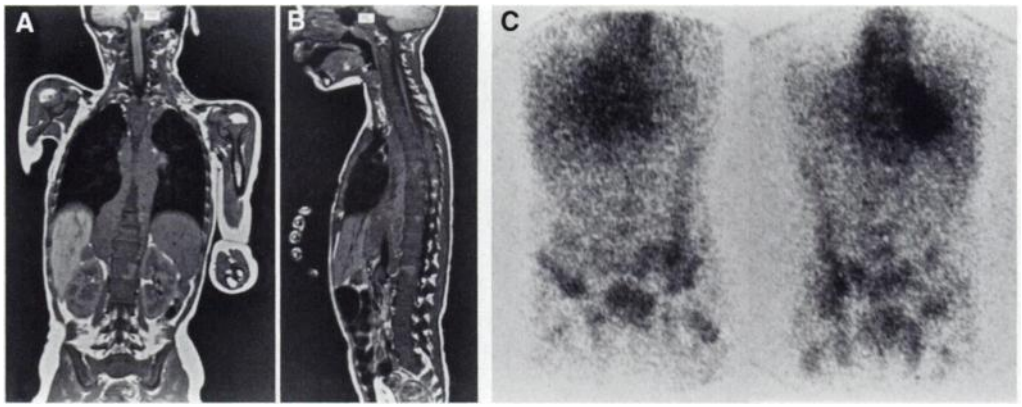

FIGURE 1. Patient 3: medullary infiltration in a 5-yr-old boy with Stage 4 neuroblastoma. (A) Coronal SE 520/15 T1W image. (B) Sagittal 520/15 T1W image. (C) A 24-hr MIBG scan with anterior (left) and posterior (right) views of abdomen and pelvis.

spine (Fig. 1A and B). The MIBG scan was concordant for a massive bone marrow involvement with an abnormal uptake in the vertebral bodies, the pelvis and upper femora. There also was high uptake in the right adrenal mass and in the bilateral paravertebral metastatic masses (Fig. 1C). Bone marrow biopsy revealed massive infiltration of the bone marrow with neuroblastoma tumor cells. Biopsy of a lymph node confirmed the diagnosis of neuroblastoma. After four courses of chemotherapy, the patient presented disappearence of bone marrow uptake of MIBG (Fig. 2). However, MRI revealed a persistent hypointense signal of the bone marrow in almost all cervical and lumbar vertebral bodies (Fig. 2), suggestive of persistent marrow infiltration, up to 27 mo after the end of chemotherapy. In this patient, trephine biopsy confirmed the absence of tumor. During a second look for residual tumor at the primary site, vertebral body biopsy of T8-T9 was performed and also was negative. Biopsy of other vertebra was not done. The clinical follow up however corroborate the results of MIBG and the patient is in complete remission.

Patient 4

A 2-yr-old boy was admitted to the hospital for abdominal pain evolving over 10 days. A painful parieto-occipital mass appeared and the boy experienced night pain in his two knees. CT and MRI revealed a retroperitoneal mass, 9 cm in diameter. Part of this mass extended into the left adrenal compartment. There was also a mass above the left clavicule and radiography of the skull showed erosion of the parietal bone. Also, MRI showed a diffuse pathological signal of the spine suggestive of diffuse bone marrow infiltration. The MIBG scan revealed a strong uptake in the retroperitoneal mass and confirmed the bone marrow metastases with diffuse abnormal uptake in the axial skeleton. The parietal mass also showed a strong uptake. Urinary catecholamines were elevated. A lymph node biopsy (subclavicular mass) confirmed the diagnosis of neuroblastoma.

After two courses of chemotherapy, MIBG scintigraphy revealed disappearance of abnormal bone uptake (Fig. 3). At the same time, marrow biopsy became negative. A persistent hypointense signal of the bone marrow of several vertebral bodies on T1WI (Fig. 3) associated with hypersignal on T2WI and Gd uptake of the same areas, was suggestive of persistent marrow infiltration. Four months after the end of chemotherapy, MRI returned to normal. The patient is in complete remission.

DISCUSSION

At diagnosis, there was concordance between MIBG scintigraphy, MRI and bone marrow biopsy for the presence of bone marrow metastasis in the four patients. Both imaging modalities showed diffuse abnormalities in three patients and focal abnormalities in the last one in whom the MIBG scan revealed an additional site of abnormal uptake in the lumbar spine. Discordance between the two imaging techniques occurred in the evaluation of the response to treatment. MRI showed two cases of false-positive results with a persistent pathological signal suggesting marrow infiltration while MIBG scintigraphy and marrow examination became negative. These two patients are in complete remission. Moreover, in one patient MRI remained falsely positive for persistent marrow infiltration up to 27 mo after the end of chemotherapy and to our knowledge, it has not been previously reported that the delay of signal normalization

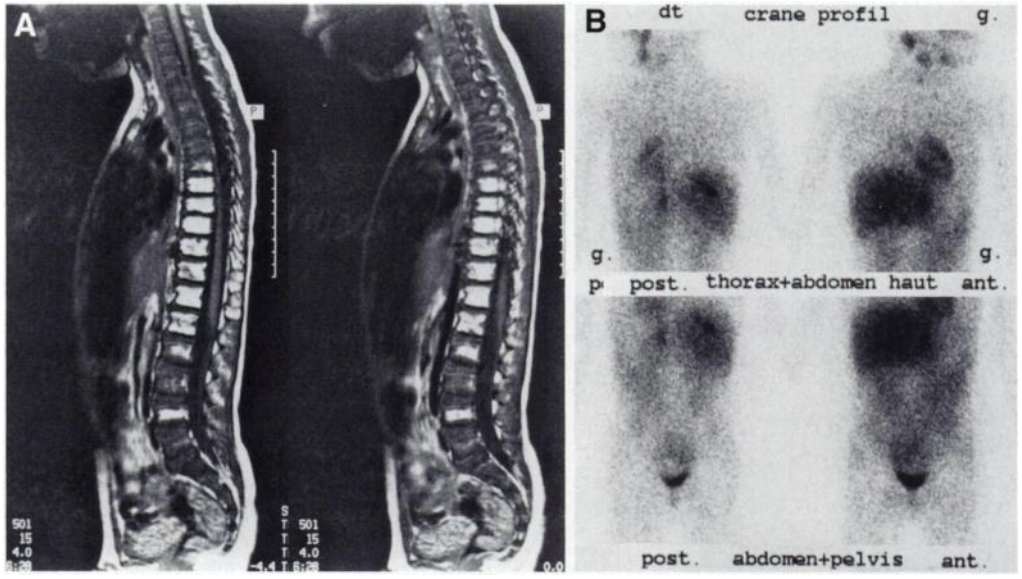

FIGURE 2. The same patient, as in Figure 1, after four courses of chemotherapy and radiotherapy of a residual paraaortic mass. (A) Sagittal SE 520/15 T1W image shows a hyperintense signal of the irradiated bodies and persistence of a hypointense signal in almost all cervical and lumbar vertebral bodies. (B) A 24-hr MIBG scan shows no uptake of the isotope in the skeleton.

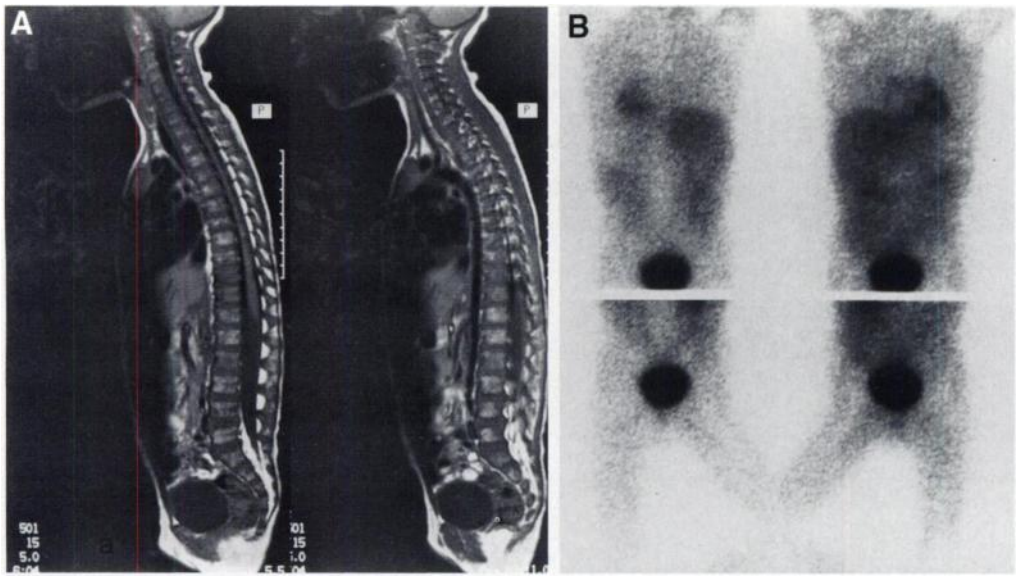

FIGURE 3. Patient 4: a 4-yr-old boy with Stage 4 neuroblastoma. (A) Sagittal SE 501/15 T1W image after two courses of chemotherapy shows a persistent hypointense signal suggesting marrow involvement in most of the vertebral bodies. (B) A 24-hr MIBG scan with posterior and anterior views of thorax and abdomen (upper row) and of the pelvis and femurs (lower row). There is no abnormal uptake in bone that could suggest the persistence of marrow infiltration.

on MRI after chemotherapy could be so long. In the second false-positive case, MRI returned to normal more quickly after chemotherapy, but again with a delay, compared to MIBG scintigraphy.

Few comparative MIBG/MRI studies exist in the evaluation of bone marrow metastasis of neuroblastoma. Tanabe et al. (*13*) compared MRI findings with histological findings in 20 patients. They histologically examined 21 specimens obtained from areas showing low intensity on T1WI images and high intensity on T2WI images, and neuroblastoma was demonstrated in 17 (81%) of them. Interestingly, they noticed that the percentage in which neuroblastoma was demonstrated varied according to the treatment state. They explained the false-positive cases with MRI by changes such as edema, bleeding and necrosis in the metastatic lesion after chemotherapy. Corbett et al. (*14*), in a prospective comparison between MRI, MIBG scintigraphy and posterior iliac crest aspiration and trephine biopsy in 30 assessments of 19 patients, have reported three patients in whom MRI was the only technique to reveal bone marrow abnormality. However, as they stated in their study, only in a minority of cases was the area of MRI abnormality biopsied, thus leaving the issue of specificity unanswered. In the study of Benz-Bohm et al. (*15*) with nine patients, MRI was more sensitive, MIBG scintigraphy and marrow aspiration more specific. Bourlière-Najean et al. (*16*), who reported the results of MRI and MIBG scintigraphy performed on the spines of 14 children with neuroblastoma, observed five cases positive for marrow metastasis by MRI and negative with MIBG. However, they obtained confirmation of marrow infiltration in iliac crest biopsy specimens in only three of five cases. Couanet et al. (*17*), in a series of 41 patients, reported the sensitvity of MRI in detecting bone marrow metastasis of neuroblastoma to be 84% and the specificity to be 88%.

Our report agrees with previous studies that also reported false-positive results with MRI in the evaluation of bone marrow metastasis of neuroblastoma. However, our cases emphasize the delay of signal normalization on MRI that can be very long (still positive MRI 27 mo after the end of chemotherapy in one patient). It seems clear that several nonmalignant conditions affect the specificity of MRI in the evaluation of bone marrow infiltration (*11,13–14*). Moreover, marrow alterations occur after chemotherapy, which also affect the specificity of MRI at reassessment after chemotherapy. Finally, the normal bone marrow signal on MRI depends on the type of bone marrow (cellular or fatty) and the localization (axial skeleton or the extremities), due to the physiological regression of red marrow to white marrow with advancing age (*11,18,19*). Ricci et al. (*19*) identified two to four major distinctive, age-related patterns of normal cellular versus fatty marrow distribution in the axial skeleton. Rests of hematopoetic tissue in areas of yellow marrow also may confuse interpretation (*11*). On the other hand, the presence or absence of bone marrow infiltration is easier to assess by MIBG scintigraphy, since normal bone/bone marrow does not accumulate MIBG. False-positive results of bone marrow involvement (diffuse uptake) have never been reported with MIBG but false-negative results do occur.

For all these reasons and since both imaging modalities are now recommended (*1*) and more frequently performed in routine practice, it is essential to use the complementarity between the functional data of MIBG and the anatomical findings of MRI to increase accuracy in staging neuroblastoma and assessing treatment response.

CONCLUSION

These cases strongly suggest that, in the presence of a complete normalization on a MIBG scan in follow up and the persistence of a pathological signal of bone marrow on MRI, attention must be paid to the delay of signal normalization, even a long time after chemotherapy, to avoid false-positive interpretation and unnecessary further therapies. Further studies are needed to understand the kinetics of bone marrow response to treatment.

REFERENCES

1. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging and response to treatment. *J Clin Oncol* 1993;11: 1466–1477.
2. Lumbroso J, Guermazi F, Hartmann O, et al. Sensibilité et spécificité de la scintigraphie à la Métaiodobenzylguanidine dans l'exploration des neuroblastomes, analyse de 115 examens. *Cancer* 1988;75:97–106.
3. Feine U, Müller-Schauenburg W, Treuner J, Klingenbiel T. Metaiodobenzylguanidine labeled with $^{123}I/^{131}I$ in neuroblastoma diagnosis and follow-up treatment with a review of the diagnostic results of the International Workshop of Pediatric Oncology held in Rome, September 1986. *Med Pediatr Oncol* 1987;15:181–187.
4. Hoefnagel CA, Voûete PA, De Kraker J, Markuse HR. Radionuclide diagnosis and therapy of neural crest tumors using iodine-131-metaiodobenzylguanidine. *J Nucl Med* 1987;28:308–314.
5. Miceli A, Nespoli L, Burgio GR, Aprile C, Carena M, Spaonaro R. The role of ^{131}I benzylguanidine in the diagnosis and follow-up of neuroblastoma. *Pediatr Hematol Oncol* 1986;3:37–47.

6. Voûete PA, Hoefnagel CA, De Kraker J. Iodine-131-meta-iodobenzylguanidine in diagnosis and treatment of neuroblastoma. *Cancer* 1988;75:107–111.
7. Bomanji J, Conry BG, Britton KE, Reznek RH. Imaging neural crest tumors with ^{123}I-metaiodobenzylguanidine and x-ray computed tomography. A comparative study. *Clin Radiology* 1988;39:502–506.
8. Müller-Gärtner HA, Ertmann R, Helmke K. Meta-iodobenzylguanidine scintigraphy in neuroblastoma. A comparison with conventional x-ray and ultrasound. *Pediatr Hematol Oncol* 1986;3:37–47.
9. Lastoria S, Maurea S, Caraco C, et al. Iodine-131-metaiodobenzylguanidine scintigraphy for localization of lesions in children with neuroblastoma: comparison with computed tomography and ultrasonography. *Eur J Nucl Med* 1993;20:1161–1167.
10. Lebtahi-HadjDjilani N, Lebtahi NE, Bischof-Delaloye A, Laurini R, Beck D. Diagnosis and follow-up of neuroblastoma by means of iodine-123-metaiodobenzylguanidine and bone scan, and the influence of histology. *Eur J Nucl Med* 1995;22:322–329.
11. Vogler JB, Murphy WA. Bone marrow imaging. *Radiology* 1988;168:679–693.
12. Cohen MD, Klatte EC, Baehner R, et al. Magnetic resonance imaging in children. *Radiology* 1984;151:715–718.
13. Tanabe M, Takahashi H, Ohnuma N, Iwai J, Yoshida H. Evaluation of bone marrow metastasis of neuroblastoma and changes after chemotherapy. *Med Pediatr Oncol* 1993;21:54–59.
14. Corbett Rolliff J, Fairley N, Moyes J, et al. A prospective comparison between magnetic resonance imaging, meta-iodobenzylguanidine scintigraphy and marrow histology/cytology in neuroblastoma. *Eur J Cancer* 1991;27:1560–1564.
15. Benz-Bohm G, Gross-Fengels W, Wiedemann B, Linden A. Knochenmarkmetastasierung bei Neuroblastom. MRT im Vergleich zur Knochenmarkzytologie und MIBG-Szintigraphie. *Fortschr Röntgenstr* 1990;152:523–527.
16. Bourliere-Najean B, Siles S, Panuel M, et al. Value of MRI and ^{123}I-MIBG sintigraphy in the diagnosis of spinal bone marrow involvement in neuroblastoma in children. *Pediatr Radiology* 1992;22:443–446.
17. Couanet D, Geoffray A. Etude en imagerie par résonance magnétique (IRM) des métastases ostéomédullaires de neuroblastomes. *Bull Cancer* 1988;75:91–96.
18. Weinreb JC. MR Imaging of bone marrow: a map could help. *Radiology* 1990;177:23–24.
19. Ricci C, Cova M, Kang YS, et al. Normal age related patterns of cellular and fatty bone marrow distribution in the axial skeleton: MR imaging study. *Radiology* 1990;177:83–88.

Manipulation of Blood Clearance to Optimize Delivery of Residualizing Label-Antibody Conjugates to Tumor Cells In Vivo

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We have attempted to improve the therapeutic index of radioimmunotherapy by manipulating the blood clearance rate and the catabolism of the radiolabel. The general strategy is to allow the antibody (Ab) to circulate in the blood for 2–3 days, then to clear it rapidly by a method that delivers the Ab to hepatocytes. In addition, the radiolabel selected has two key properties: it is a residualizing label (which is lysosomally trapped after catabolism), so it is retained well by tumor cells, but is excreted rapidly by hepatocytes into bile. **Methods:** In initial experiments, three residualizing radiolabels were tested for their rate of excretion after specific delivery in vivo to either hepatocytes, via galactosylated Ab, or Kupffer cells, via immune complexes. A label showing rapid biliary excretion only after delivery to hepatocytes, ^{111}In-benzyl-diethylenetriamine tetraacetic acid, was then used for radioimmunodetection in a protocol of delayed rapid blood clearance in which clearance was by hepatocytes. This was achieved by using galactosylated Ab, combined with temporary inhibition of the asialo-glycoprotein receptor on hepatocytes. Ab RS11 and the lung adenocarcinoma Calu-3 xenograft in nude mice were used. Control experiments were performed with a conventional ^{125}I label and with ^{125}I-dilactitol-tyramine. **Results:** Indium-benzyl-diethylenetriamine tetraacetic acid was identified as a label that was excreted more rapidly from hepatocytes than from Kupffer cells, by biliary excretion. Using this radiolabel with delayed rapid blood clearance, very high tumor/blood ratios were obtained, 166:1 at day 3, but tumor/normal tissue ratios for other tissues were not as high. There appeared to be some uptake of the radiolabel by all normal tissues tested, including the lungs and muscle. Dosimetry calculations suggested that the therapeutic index was no better than with a simple Ab injection. **Conclusion:** Antibody catabolism can be directed towards either hepatocytes or Kupffer cells, and this difference can strongly affect the excretion rate of radiolabels, since only hepatocytes can excrete degradation products into bile. Processing will also depend on the particular radiolabel. These factors are particularly important for protocols involving delayed rapid blood clearance, since liver uptake is so rapid. The methods described should stimulate other approaches of manipulating Ab blood clearance and radiolabel catabolism to achieve improved therapeutic results.

Key Words: antibody localization to tumors; residualizing radiolabels; rapid blood clearance of antibodies; antibody catabolism in liver

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Use of antibody (Ab)–radioisotope conjugates to specifically deliver radiation to tumors has been the focus of many experimental studies, with some recent encouraging clinical results (*1*). The most widely used strategy is the simplest approach; namely, injecting radiolabeled Abs. However, there are many possible methods of modifying this basic approach with the aim of increasing the therapeutic index. For the reasons described below, we have combined three modifications: (a) induction of delayed rapid blood clearance of the Ab after 2–3 days of circulation (slow clearance followed by rapid clearance), (b) use of a residualizing radiolabel (a label which is trapped within the cell after catabolism of the Ab to which it was originally conjugated) and (c) clearance by hepatocytes rather than macrophages and Kupffer cells.

Rationale for Delayed Rapid Blood Clearance

The major toxicity resulting from radioimmunotherapy is myelotoxicity, resulting primarily from the radiation dose delivered by circulating Ab to the bone marrow. Because Abs bind specifically at the tumor site, and because blood clearance of immunoglobulin G (IgG) is quite slow, it seems useful to induce rapid blood clearance after the tumor has been maximally penetrated by the Ab, which may be expected to take 2–3 days (*2,3*). This would be particularly advantageous if saturating levels of Ab have been used, since no further binding to the tumor cells can occur. Delayed rapid blood clearance has been

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