In Vitro and Animal Validation of Bromine-76-Bromodeoxyuridine as a Proliferation Marker

Mats Bergström, Li Lu, Karl-Johan Fasth, Feng Wu, Erzsebet Bergström-Pettermann, Vladimir Tolmachev, Elisabeth Hedberg, Aiping Cheng and Bengt Långström

Subfemtomole Biorecognition Project, Japan Sciences Technology Corporation and Uppsala University PET Centre, University Hospital, Uppsala, Sweden

The potential of ^{76}Br-bromodeoxyuridine as a PET tracer for characterizing proliferation potential was investigated in multicellular tumor aggregates and in healthy rats and pigs. **Methods:** Bromine-76-bromide was produced by proton irradiation of a ^{76}Se-enriched target using a 17-MeV cyclotron and recovered by thermal diffusion. Bromine-76-BrdU was prepared from the corresponding trimethylstannate by an oxidative bromination. Multicellular aggregates from a carcinoid cell line and two bladder cancer cell lines were coincubated with ^{76}Br-BrdU and ^{3}H-thymidine and the uptake and DNA incorporation analyzed. About 0.5 MBq ^{76}Br-BrdU were injected in the tail vein of unanaesthetised Sprague-Dawley rats. Two to 36 hr later they were decapitated and the radioactivity concentration and fraction of radioactivity incorporated into DNA determined in five different organs and the blood. Parallel studies were performed in animals pretreated with hydroxyurea. In separate experiments, rats were given an injection of ^{76}Br-bromide and organ uptake was evaluated after 20 hr. PET studies were performed in two pigs and the uptake in different organs was investigated after injection of ^{76}Br-BrdU. In these studies, diuresis was induced by furosemide and mannitol and radioactivity in blood and organs was followed during 10 hr. **Results:** In the cell aggregates, 30%–90% of the radioactivity was extracted in the DNA fraction. A good correlation was found between ^{76}Br-BrdU and ^{3}H-thymidine with respect to total uptake and DNA fraction. The DNA fraction increased from 2–10 hr after incubation. With in vivo injection in the rat, relatively high uptake of radioactivity was found in all organs, unrelated to the degree of DNA synthesis. However, inhibition by hydroxyurea occurred only in the spleen and intestines, organs which also showed a high degree of incorporation of ^{76}Br-BrdU into DNA. In the pig, the highest in vivo uptake was observed in the red bone marrow and the intestines. In these organs, 70%–80% of the radioactivity was recovered in the DNA fraction. The concentration of radioactivity in the heart, liver and kidney was 3–10 times lower, and here the DNA fraction accounted for 10%–20% of the radioactivity. The decay-corrected radioactivity in blood and nonproliferating organs decreased with diuresis with a half-life of 13 and 16 hr, respectively. **Conclusion:** It is suggested that the radioactivity uptake as seen after the administration of ^{76}Br-BrdU, is constituted by two parts: one relating to incorporation into DNA and one existing as free $^{76}Br^-$ or metabolites of ^{76}Br-BrdU. If sufficient time has passed, $^{76}Br^-$ dominates other metabolites. A correct assessment of DNA-incorporated radioactivity using PET with ^{76}Br-BrdU is not trivial and can only be made with due correction for $^{76}Br^-$, using either a complementary investigation after hydroxyurea pretreatment (in animal studies) or a separate ^{76}Br-bromide investigation. Alternatively, the free bromide can be eliminated partially through forced diuresis.

Key Words: PET; bromodeoxyuridine; cell proliferation; DNA synthesis; radioisotopes

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PET has been used extensively to characterize tumors since its development in the 1970s. Areas to investigate have been whether this technique could supply better methods for diagnosis or for malignancy grading. Although a large repertoire of tracer substances has been used in oncology, only ^{18}F-fluorodeoxyglucose and ^{11}C-methionine have been used to the extent that their roles in clinical practice can be established. These tracers indicate glucose metabolism and amino acid transport, respectively. These are parameters that have been demonstrated to be enhanced in many tumor types, thus allowing identification of tumors; enhanced in relation to malignancy grade within the same tumor type; and sensitive indicators of response to treatment.

These features are, however, not without controversy and cellular metabolism in these respects has no simple relation to malignant features such as proliferation and tumor growth. It is fair to say that over a large spectrum of tumor types, enhanced metabolism of glucose or amino acids is not well correlated to more direct immunohistochemical measures of cell proliferation such as staining for ^{67}Ki or BrdU (5-bromodeoxyuridine).

Several attempts have been made to characterize proliferation potential in vivo with PET using ^{11}C-thymidine, labeled either in the methyl group or in position two in the ring (*1–8*). Although in some instances correlation with malignancy has been found, it seems that in-between tumor types, no consistent pattern of enhanced tracer uptake in relation to malignancy is found. It is likely that the uptake of this tracer primarily is reflecting factors such as tissue perfusion and expression of the nucleoside transporter. The PET radioactivity signal is probably reflecting primarily intracellular metabolites of thymidine, including the phosphorylated forms, for this tracer. The time window allowed by the half-life of ^{11}C seems too short to allow elimination of these metabolites from the cells and to leave a dominance by radioactivity incorporated into DNA.

It may be possible to extend the observation period by using ^{76}Br, which has a half-life of 16 hr and can be used to label the thymidine analog bromodeoxyuridine using ^{76}Br-bromide in an oxidative bromination of the corresponding trimethylstannate (*9*). Recent developments in the production of ^{76}Br through the ^{76}Se(p,n) ^{76}Br reaction using a low-energy accelerator (17-MeV protons) followed by thermal-diffusion radionuclide separation (in preparation), has allowed a routine production of ^{76}Br, a prerequisite for a clinical use of this radionuclide (Tolmachev V, *personal communication*, 1998).

Bromodeoxyuridine (BrdU) is well characterized and used extensively for immunohistochemical assessment of proliferation potential on operative samples (*10,11*). It is transported across the cell membrane with the same transporter as thymidine, rapidly phosphorylated and incorporated into DNA with a similar affinity as thymidine (*12,13*). Several publications have previously indicated ^{77}Br- or ^{82}Br-labeled BrdU or ^{123}I- or ^{131}I-labeled IdU (iododeoxyuridine) as potential tracers for DNA synthesis (*14–25*) as well as for targeted radiotherapy in oncology. Although the use these analogs for imaging appears

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For correspondence or reprints contact: Mats Bergström, Uppsala University PET Centre, University Hospital, S-751 85 Uppsala, Sweden.

attractive, so far they have been used with limited success. The primary obstacle has been a short biological half-life (5–7 min in humans), inducing a massive release of the free labels (^{77}Br or ^{123}I) into the circulation and in physiological secretions (salivary glands, gastrointestinal tract, kidney/urinary tract). A careful study by Kriss et al. (*14*) indicated that 10 min after administering ^{82}Br-BrdU, 90% of the radioactivity in plasma was constituted by ^{82}Br-bromide.

In spite of previous obstacles with using radiolabeled BrdU, we wished to explore the potential of ^{76}Br-BrdU as an in vivo tracer of DNA synthesis. We hoped that, with a longer follow-up time, the quantitation accuracy and tomographic imaging of PET and methods to reduce the influence ^{76}Br-bromide would solve some of the problems. We initiated this series of experiments in cell culture and in vivo experiments in rats and pigs to study this.

MATERIALS AND METHODS

Chemistry

Bromine-76-Br$^-$ was produced using the Scanditronix MC-17 cyclotron at the Uppsala University PET center using the ^{76}Se(p,n)^{76}Br nuclear reaction by proton irradiation of [^{76}Se]Se-enriched Cu_2Se (96.5% enrichment). The [^{76}Br]-Br was separated from the Cu_2Se pellet with a thermal diffusion procedure (Tolmachev V, *personal communication*, 1998).

The precursor 5-trimetylstannyl-2′-deoxyuridine, used in the synthesis of 5-[^{76}Br]-bromo-2′-deoxyuridine, was synthesized from 5-iodo-2′deoxyuridine according to published procedures (*26–28*).

The synthesis of 5-[^{76}Br]-bromo-2′-deoxyuridine was performed in a semiautomated mode including preparative HPLC purification in our robotic system for radiopharmaceutical production, SYNTHIA (*29*). The process was controlled by a programmable logical controller (GE Fanuc, Automation, Inc., Charlottesville, VA) in conjunction with a laboratory robot (Gilson ASPEC, Villiers-le-Bel, France), both interfaced to a user interface (Wonderware InTouch, Wonderware Corp., Irvine, CA) allowing recipe handling. Thus, a standard file was created for 5-[^{76}Br]-bromo-2′-deoxyuridine ensuring high batch-to-batch reproducibility.

To a solution of 5-trimetylstannyl-2′-deoxyuridine (1–2 mg) in 1% acetic acid in ethanol (140 μl), was added the [^{76}Br]-Br$^-$ in ethanol (200 μl) followed by a chloramine-T solution in ethanol (2.2 mg/ml; 60 μl). The mixture was heated at 60°C for approximately 20 min, diluted with saline and purified by preparative high-performance liquid chromatography (HPLC) on a Beckman Ultrasphere C-18 column (10 × 250 mm). Sterile saline with 5% ethanol and a gradient to 10% ethanol during 5 min was used as the mobile phase and the flow was 7 ml/min. The fraction eluting with a retention time of about 8 min was collected and sterile filtrated. Radiochemical purity and identity were assessed using analytical HPLC with addition of authentic reference material. A Beckman Ultrasphere C-18 column (4.6 × 250 mm), water/acetonitrile (95/5 gradient to 92/8 during 8 min) as mobile phase and a flow of 2 ml/min was used. The retention time of BRU was 6 min. Thin-layer chromatography on Alugram Sil G/UV$_{254}$ silica plates using chloroform/methanol (90/10) with an R_f value of 0.24 for BRU was used also to analyze radiochemical purity and identity.

Cell Culture

Cell cultures were established from a human carcinoid tumor cell line (BON-I; Dr. CM Townsend, University of Texas, Galveston, TX) (*30*), grown in Ham F-12K medium containing L-glutamic acid 29.4 mg/ml) (NordCell, Sweden) mixed with DMEM medium without L-glutamic acid (1:1) (Seromed, Germany), and two bladder cancer cell lines, RT4 and J82 (American Type Culture Collection, MD), cultured in MEM-Eagle medium (containing L-glutamic acid 14.7 mg/ml). Both media were supplemented with 10% fetal bovine serum, 2 m*M* L-glutamine, penicillin (100 IU/ml) and streptomycine (100 μg/ml) (Seromed, Biochrom KG, Germany). The culture media contained thymidine at a concentration of 0.7 mg/l. Agarose-coated well plates were generated by depositing 0.5 ml of a 1% agarose solution in the bottom of each well of the plate (*31,32*). Before use, these agarose-coated wells were washed three times with fresh medium. The tumor cells were harvested from the stem monolayer culture after digestion with trypsine supplemented with EDTA. Cell suspensions were seeded in 24-well culture plates with 10.000 cells per well for BON and 50.000 cells per well for RT4 and J82. The cells cultures were kept at 37°C with 5% CO_2. The culture medium was changed once every 2 days beginning 4 days after seeding. The aggregates were ready for experiments from the fifth day after seeding.

Ten to 12 aggregates were collected in one well with 1 ml of medium for the incubation with typically 0.25 MBq ^{76}Br-BrdU (specific activity 1.86 GBq/μmol) plus 37 kBq ^{3}H-thymidine (specific activity 247 GBq/mmol) for 2 hr. A separate group of aggregates was given 5 m*M* of hydroxyurea, in conjunction with the radioactivity, to selectively block the replication DNA synthesis (*33*). After the incubation, the aggregates were moved three times between wells without radioactivity to wash away any nonincorporated tracer. The aggregates were collected for DNA separation and analyzed at 0, 2, 14 and 22 hr after the end of incubation. Bromine-76 radioactivity was measured in a well counter and ^{3}H 3 days later in a scintillation counter. The total uptake, in relation to the similar volume of incubation medium and the DNA fraction of radioactivity, was compared between ^{76}Br and ^{3}H. Each experiment with a specific cell line and time point was made in duplicate.

Rat Studies

Sprague-Dawley rats about 12 wk old and with a body weight of about 400 g were used in the experiments. The studies were accepted by the local research animal ethics committee (permission C 184/95). The rats were allowed free access to water and food before and during the entire experimental period. In unanaesthetised conditions, the rats were given a bolus injection in the tail vein with 0.5 MBq ^{76}Br-BrdU in 0.7 ml saline. In separate experiments, the rats were given a dose of 200 mg hydroxyurea intravenously 30 min before injection of the radioactivity. At 2, 4, 8, 16 and 32 hr after administering the radioactivity, the rats were sedated by CO_2-inhalation and decapitated. Samples of blood, liver, lung, kidney, heart, spleen and intestines were taken and their weight and radioactivity were measured. A 20-μl sample of the injection solution also were measured for radioactivity. The radioactivity concentrations of the organs were calculated and presented as standardized uptake values (SUVs) by dividing the radioactivity concentration by the ratio of total given radioactivity and body weight. This standardization compares the organ radioactivity concentration to an assumed equal total-body concentration. As a further standardization, the organ radioactivity concentration was given in relation to whole-blood radioactivity concentration. Each time point was repeated in a minimum of four and up to eight rats and the averages and standard deviations calculated.

Separate samples of the organs were processed further to determine the fraction of radioactivity incorporated into DNA (see below). For each time point, four to eight rats were used. The averages and standard deviations of SUV, ratio to blood and DNA-incorporated fraction were calculated. In a separate experiment, rats were given an injection of 0.5 MBq ^{76}Br-bromide and 20

hr later the animals were killed and organ radioactivity determined as above.

Pig PET Studies

Two Göttingen minipigs, weighing 27 and 30 kg, were used in separate experiments and anaesthetised with pavulon 0.26 mg/kg/hr and pentobarbital 8 mg/kg/hr and intubated for artificial ventilation. These studies were performed under the permission by the local research animal ethics committee (permission C 63/96). The pig was placed on the couch of the PET camera and three different anatomical sectors were indicated: the liver-spleen, lungs and lower abdomen including intestines. Each sector was covered by 15 slices with a slice separation of 6.5 mm and an in-plane resolution of 5.5 mm (*34*). Transmission scans were made over the three sections using an external rotating ^{68}Ge rod. An initial dynamic imaging sequence over the liver was started after 37 and 47 MBq ^{76}Br-BrdU was given as a rapid bolus injections. This sequence consisted of 16 time frames aquired during 65 min. Diuresis was initiated then with furosemide 1 mg/kg/hr and mannitol 200 mg/kg/hr combined with an infusion of saline-dextrose. Repeat 10-min scans were made once every hour at each of the sectors for 10 hr. Blood samples were taken twice per hour and analyzed for whole-blood and plasma radioactivity concentration.

In the PET images, regions of interest were outlined to represent liver, lung, heart, muscle, spleen, intestines and red bone marrow. The radioactivity concentration in each region and each time frame was measured and recalculated to SUVs by dividing the concentration by the ratio of the given radioactivity and body weight. Time-activity curves of the various organs and blood were plotted.

DNA Separation and Analysis

The aggregates, or 100 mg from the tissue samples, were added to 1 ml of DNAzol (Life Technologies, NY) and homogenized using a polytron homogenizer. The homogenate was divided in two, of which one part was centrifuged and the band containing the DNA fraction was collected. Ethanol was added to the DNA fraction to precipitate the DNA. The tube was centrifuged, the supernatant taken off and the pellet was washed once more with ethanol followed by a renewed centrifugation. The pellet, representing DNA, and half of the homogenate, representing total radioactivity, was measured with regard to radioactivity and the DNA-incorporated radioactivity was calculated as a percentage of total radioactivity.

In a separate sample, the amount of free ^{76}Br-bromide was determined by precipitation with $AgNO_3$. Briefly 200 mg of tissue was homogenized in 2 ml 100 m*M* NaCl. After centrifugation at 3000 rpm in 10 min, the supernatant was removed. The bromide and chloride were precipitated with 100 m*M* $AgNO_3$. After centrifugation for 5 min at 15,000 rpm, the pellet was resuspended and reprecipitated. The final pellet was measured and radioactivity was expressed as percent of original sample radioactivity.

RESULTS

Chemistry

The production of ^{76}Br through the ^{76}Se(p,n) ^{76}Br reaction using a low-energy accelerator (17 MeV protons and 10–14 μAh beam current) followed by thermal-diffusion resulted in about 65–70 MBq/μAh using a pellet of Cu_2Se (180 g/cm2 (96.5% enrichment in ^{76}Se).

Using dry distillation and a thermal chromatographic procedure with argon gas ^{76}Br-bromide was recovered in 65%–75% within 60–75 min (Tolmachev V, *personal communication*, 1998). The radioactive bromide was obtained after washing the collecting tube with 200 ml ethanol. The radiochemical purity was more then 95% and radionuclide purity was more than 98%.

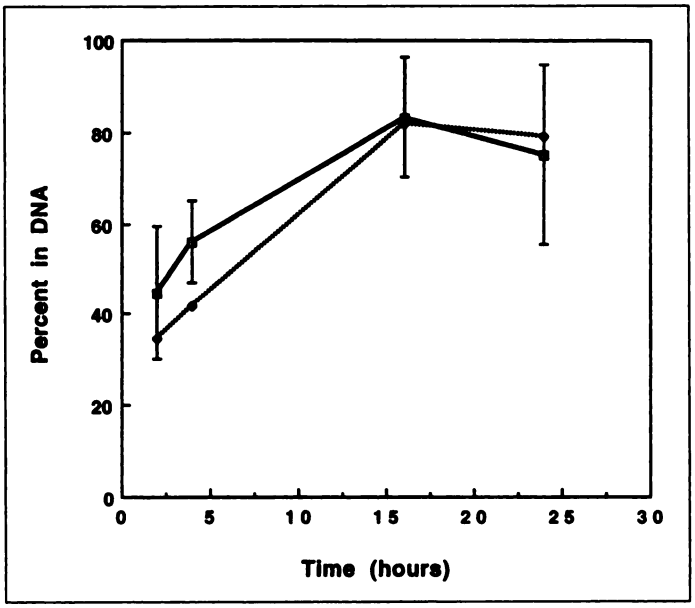
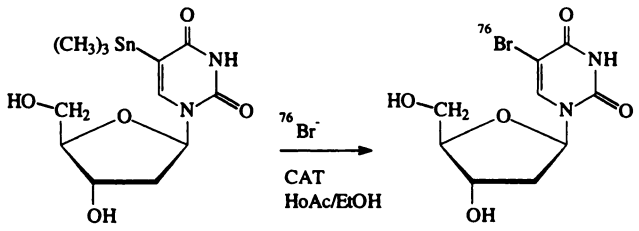


FIGURE 1. Percentage of radioactivity recovered in DNA fractions in multicellular aggregates exposed to ^{76}Br-BrdU (continuous line) or ^{3}H-thymidine (broken line) for 2 hr followed by further incubation up to 24 hr without radioactivity (1 s.d. indicated).

The 5-[^{76}Br]-bromo-2′-deoxyuridine was prepared from 5-trimetylstannyl-2′-deoxyuridine by an electrophilic substitution reaction using chloramine-T as an oxidizing agent:



The incorporation of ^{76}Br was almost quantitative and the product was isolated in approximately 60% radiochemical yield and > 99% radiochemical purity within 40 min from the start of synthesis. The specific activity was approximately 2 GBq/μmol.

Cell Culture

The total uptake in cell aggregates was similar for ^{76}Br-BrdU or ^{3}H-thymidine, with a correlation coefficient of 0.91. The fraction of ^{76}Br-BrdU incorporated into DNA was increasing from about 60% at the end of the 2-hr incubation time to 80% after a further 14 hr without radioactivity in the BON aggregates and from 40% to 80% in RT4 and J82 aggregates. The total radioactivity decreased with time, while the portion in DNA was constant. There were no differences between the DNA fractions when using ^{76}Br-BrdU and ^{3}H-thymidine when compared within one cell line. To illustrate the general trends, the data were averaged over the three cell lines used (Fig. 1). When the data for these two tracers were compared in each individual experimental point, an excellent correlation was noted (R2 = 0.83) (Fig. 2; one data point lost for technical reasons). When hydroxyurea was given simultaneously with the radioactivity, the amount incorporated into DNA was reduced by 80%–90% both for ^{76}Br-BrdU and ^{3}H-thymidine.

Rat Studies

In the rats given only a tracer dose of ^{76}Br-BrdU, the highest uptake was found in the spleen and intestines, closely followed

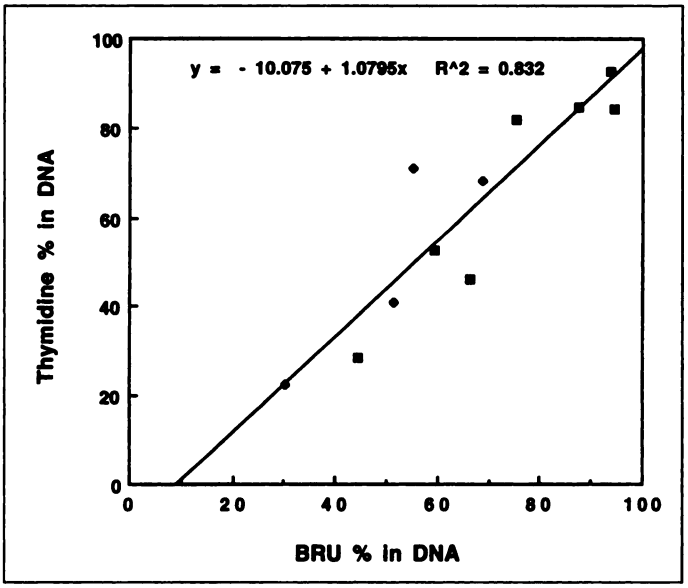


FIGURE 2. Correlation between DNA fractions of radioactivity after incubation of multicellular aggregates with ^{76}Br-BrdU or ^{3}H-thymidine.

by whole blood, kidney and lung. The heart and liver uptake was about half that of the other organs. No clear temporal pattern could be distinguished, but the radioactivity uptake seemed constant with time. The variations between the different time points was of the order of 20%. In the rats pretreated with hydroxyurea, the radioactivity was lower by about 50% in the spleen and intestines compared to the tracer study. The kidney uptake was about 20% lower, whereas no difference could be seen in the heart, liver, lung or blood. When the organ radioactivity was represented as a fraction of blood radioactivity (Fig. 3) the variations between the time points were significantly reduced. Again no temporal variation could be seen, but a pronounced difference was found between the tracer group and the group pretreated with hydroxyurea for intestines and spleen, although no difference was observed in heart, lung and liver.

The analyses of the fraction of radioactivity incorporated into DNA, showed about 50% DNA incorporation into the spleen and intestines, whereas in the liver and heart it was about 5% (Fig. 4). With hydroxyurea pretreatment the DNA-incorporated fraction in spleen and intestines decreased to about 5%. A significant correlation was observed between the fractional DNA incorporation for the different organs and the percent reduction induced by hydroxyurea. The DNA-incorporation fraction was quantitatively the same as the reduction induced by hydroxyurea.

In the experiments performed with the injection of ^{76}Br-bromide, the highest concentration was found in whole blood followed by the lungs. The concentration in the other organs was 40%–60% of the blood concentration. A significant correlation was noted between the organ uptake of ^{76}Br-BrdU after hydroxyurea pretreatment and the organ uptake of ^{76}Br-bromide (Fig. 5).

Pig PET Studies

The images and values obtained in the two pigs were similar. The initial images showed rather uniform uptake in the various organs with slightly higher values in the liver. After a few minutes and until the end of the study, the highest uptake was noted in the red bone marrow, followed by the intestines (Fig. 6). The intestine and red bone marrow uptake were constant with time from about 50 min after injection, whereas the uptake in lung, heart, liver and muscle decreased throughout the study (Fig. 7). The uptake in blood was higher initially than in any organ (SUV about 1.2), but decreased to be lower than the red bone marrow (SUV = 0.6) at the end of the study. After the first few minutes, the blood radioactivity decreased exponentially with a half-life of 16 and 13 hr in the two pigs. The plasma/whole-blood ratio was initially 1.25 and increased with time to 1.4.

Samples of heart, liver, lung, kidney, spleen, red bone marrow and intestines were taken from both pigs after they

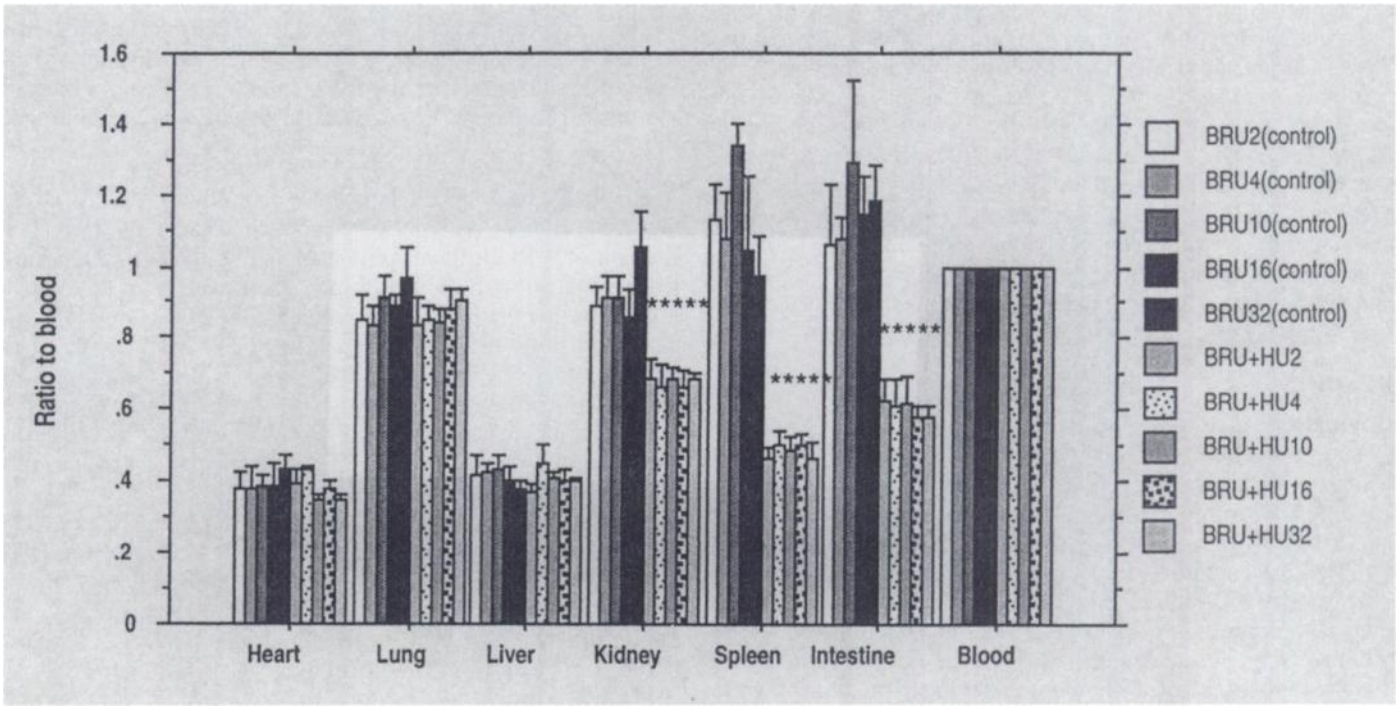


FIGURE 3. Organ radioactivity concentration in rats given intravenous injection of ^{76}Br-BrdU. Uptake expressed as a ratio to blood radioactivity concentration. Animals were decapitated after 2, 4, 8, 16 or 32 hr. Separate animals were given 200 mg hydroxyurea 30 min before injection of radioactivity. Each bar represents the average of four to eight rats. Stars indicate statistically significant differences between tracer and tracer plus hydroxyurea study.

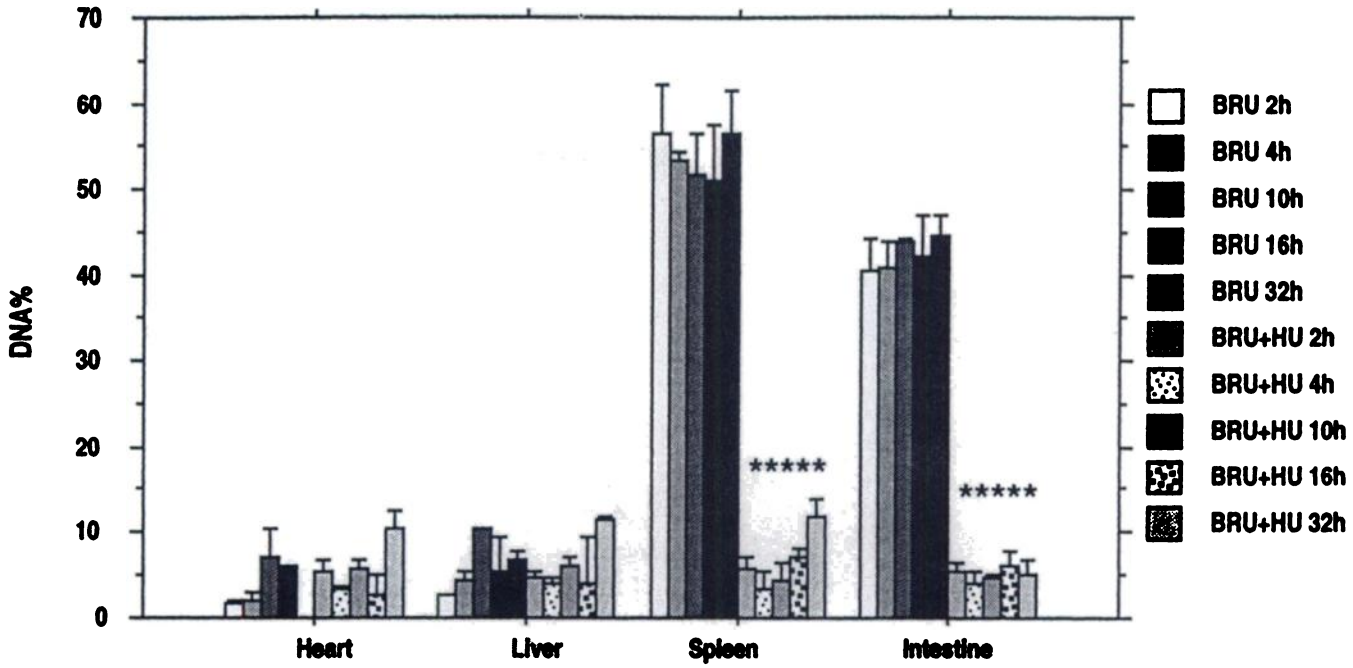


FIGURE 4. Fraction of organ ^{76}Br-BrdU radioactivity recovered in the DNA fraction in various organs.

were killed. These samples were analyzed for fraction of DNA-incorporated radioactivity. The red bone marrow showed the highest amount of total radioactivity as well as the highest degree in the DNA fraction, 84% (Table 1), followed by spleen, intestines and lung, in that order. Low total uptake and DNA fractions were found in heart, liver and kidney. The radioactivity concentration in the blood was high, but basically all of that was recovered in the bromide fraction.

DISCUSSION

Parallel studies performed with ^{76}Br-BrdU and ^{3}H-thymidine in multicellular aggregates indicate that ^{76}Br-BrdU behaves similarly to ^{3}H-thymidine with respect to degree of uptake and rate of incorporation into DNA. This is encouraging for the potential use of ^{76}Br-BrdU as a proliferation marker. These studies also indicate that it might be necessary to wait up to 10 hr to ensure that the radioactivity as measured will represent primarily radioactivity incorporated into DNA. We have seen previously that tumor cells cultured as multicellular aggregates have a much lower DNA incorporation rate, as compared to the exaggerated growth in a monolayer culture when cellular contact inhibition is not present (Bergström M, *personal communication*, 1991). This is one reason why we have preferred to use this cell culture mode.

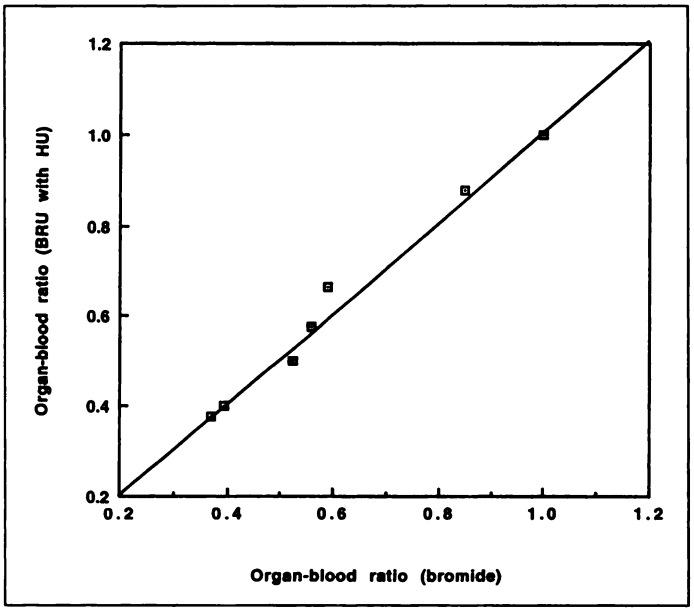


FIGURE 5. Correlation between relative concentration of radioactivity in rat organs after pretreatment with hydroxyurea and injection of ^{76}Br-BrdU, and relative concentration of radioactivity after injection of ^{76}Br-bromide.

From the present in vivo experiments, it seems clear that the quantitative uptake of radioactivity in an organ alone, after administration of ^{76}Br-BrdU, is not indicative of the degree of DNA synthesis. For example, in the rat a high uptake was noted in the lung and a rather high uptake in the liver and heart, organs known to have a low proliferation. These relationships did not change even with follow-up times up to 36 hr after administration of the tracer. This indicates that the preliminary hope is not justified, that a long waiting time would allow intact ^{76}Br-BrdU and its metabolites to be cleared from the tissues and that the residual radioactivity would represent ^{76}Br-BrdU incorporated into DNA. However, when a separate examination was made after administering hydroxyurea, an agent known to block the replication DNA synthesis (*33*), a distinct pattern of inhibition was observed in proliferating tissues. The degree of inhibition of uptake correlated with the amount of radioactivity bound in the DNA in the same organs. Thus the spleen and intestines, organs expected to have the highest degree of DNA synthesis, both had a 50% inhibition by hydroxyurea and 50% of the radioactivity incorporated into DNA. The organs with minimal or no effect by hydroxyurea also had a minimal degree of DNA incorporation. A small residual radioactivity of about 5% in the DNA fraction in these latter organs probably reflects a slight contamination by low-molecular radioactive molecules.

These data indicate that it should be possible to use ^{76}Br-BrdU to assess proliferation potential if the tracer study is

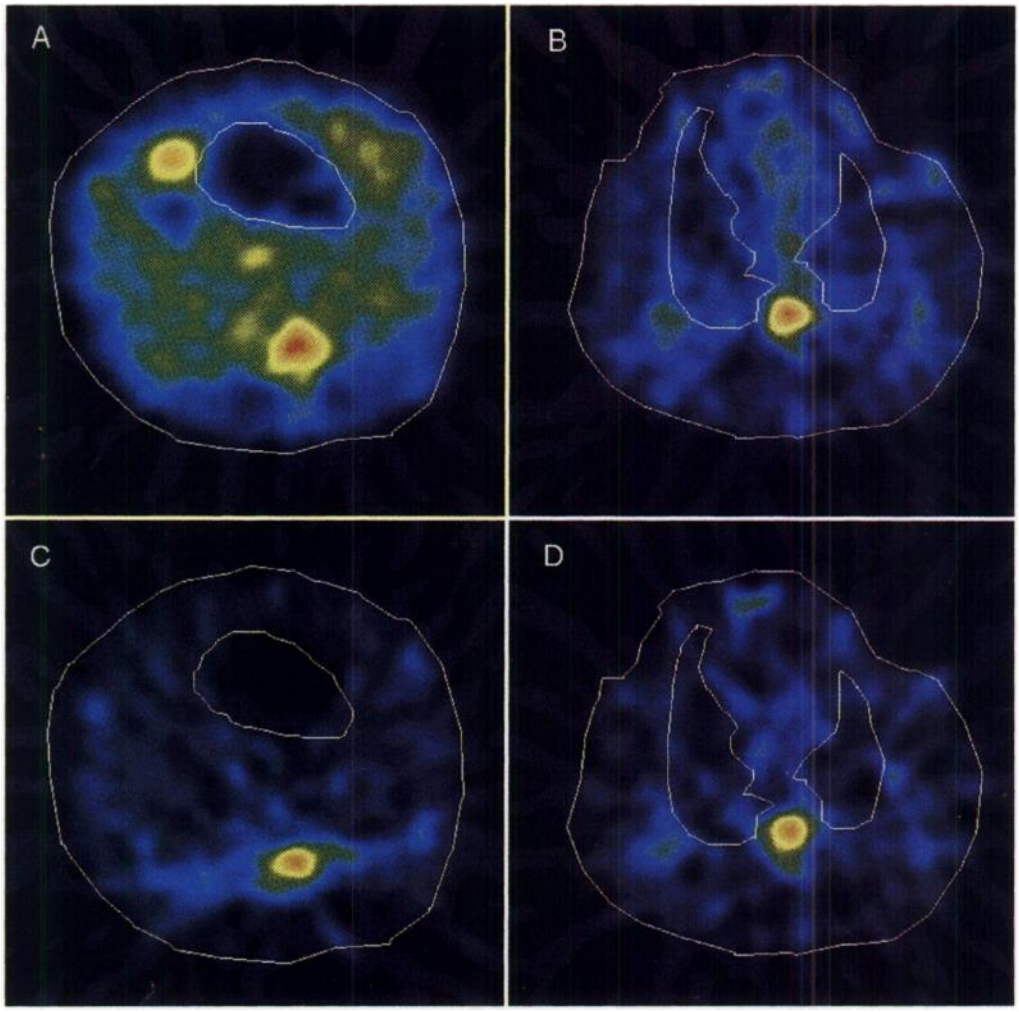


FIGURE 6. Images over the liver (left images) and lung (right images) in the pig after injection of ^{76}Br-BrdU. Upper row, images obtained 1 hr after injection; bottom row, images obtained 10 hr after injection.

complemented by a repeat study made after administering hydroxyurea, or as indicated by old data, after administering Ara-C (*33*). This is not applicable to humans, but it could be used in animal experiments.

The fact that a large residual part of radioactivity remains in all organs, even after long follow-up times, indicates that this radioactivity must be in a chemical form that does not readily leave the body. A plausible suggestion is that it is constituted by ^{76}Br-bromide. Kriss et al. (*14*) have demonstrated that the major portion of radioactivity in plasma in humans is constituted by ^{82}Br-bromide after administering ^{82}Br-BrdU. Bromide is known to have a long elimination half-life from the body of about 10 days in normal individuals (*35*). Furthermore, bromide is known to distribute almost exclusively in the extracellular fluid compartment. In fact bromide has been used extensively to trace the total-body extracellular volume (*36*). The cell culture studies indicate that metabolites of ^{76}Br-BrdU are not remaining intracellularly if more than 10 hr elapses.

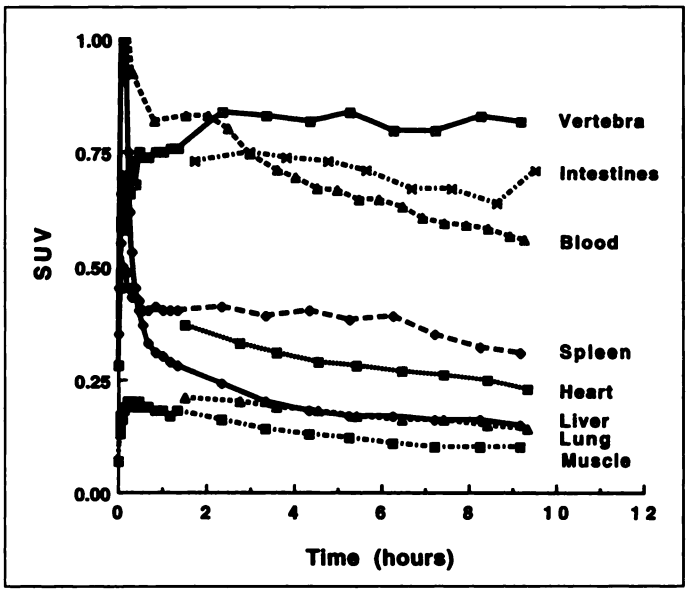


FIGURE 7. Radioactivity concentration in blood and organs of the pig, expressed as SUV, plotted against time after injection of ^{76}Br-BrdU.

When the organ distribution was evaluated after administering ^{76}Br-bromide, the relative and absolute uptake values were almost identical to those observed with ^{76}Br-BrdU after pretreatment with hydroxyurea. This, in turn, indicates a possibility to evaluate the DNA-associated radioactivity by applying a subtraction of nonspecific organ radioactivity in a ^{76}Br-BrdU investigation, using data from a separate ^{76}Br-bromide study.

A third possibility to eliminate the disturbing influence of non-DNA-associated radioactivity, presumably being bromide, is through forced diuresis. Ellenhorn and Barceloux (*37*) have reviewed the literature on bromide intoxication and write that the elimination half-life of bromide can be reduced down to 1.6 hr using the combination of ethacrynic acid and mannitol. In the separate investigations performed in pigs, such a diuresis was attempted. Although a strong diuresis was induced, resulting in more than 3 liters of urine excreted, only about a 50% reduction in blood radioactivity could be attained during 10 hr. However, in nonproliferating organs the same degree of reduction was

TABLE 1
Total Radioactivity and DNA and Bromide Fractions Determined in Pig Organs 10 Hours After Administration of Bromine-76-BrdU and with 8 Hours of Diuresis*

Organ	Radioactivity concentration (Bq)	DNA-associated radioactivity (Bq)	DNA fraction (%)	Bromide fraction (%)
Heart	61	6	12	88
Liver	68	14	21	75
Kidney	98	7	8	—
Lung	223	129	57	68
Spleen	437	350	81	44
Intestine	336	226	68	48
Bone marrow	623	526	84	—
Blood	541	8	1	92
Urine	—	—	—	100

*Organ radioactivity and DNA fractions based on experiments in two pigs and bromide determination in one pig.

observed, pointing to the feasibility of the concept. In proliferating organs, such as red bone marrow, spleen and intestines, no reduction was seen. If the reduction of free ^{76}Br-bromide in organs parallels the reduction measured in blood, it should be possible with simple arithmetic to calculate the fractions of organ radioactivity constituted by ^{76}Br-bromide and ^{76}Br-BrdU incorporated into DNA when complete elimination is not achieved by diuresis.

CONCLUSION

Bromine-76-BrdU might allow a determination of proliferation potential in vivo using PET, but only with due consideration to a high nonspecific organ radioactivity constituted by ^{76}Br-bromide. An estimate of this confounding factor can be made with a complementary investigation after hydroxyurea pretreatment (applicable only to animal studies) or with a separate ^{76}Br-bromide study to allow the subtraction of the nonspecific organ uptake which is large. If a forced diuresis could eliminate significant amounts of ^{76}Br-bromide from the tissues, however, a single study with ^{76}Br-BrdU might be sufficient.

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