Radiol Oncol 1996; 30: 249-54.

Technetium labeled autologous polyclonal immunoglobulin G (IgG) for scintigraphy of inflammation

Nataša V Budihna, Silvester Kladnik, Tomaž Benulič[1], Metka Milčinski

Nuclear Medicine, University Medical Center, [1]Institute of Oncology, Ljubljana, Slovenia

Background: Radiolabeled heterologous polyclonal human gamma globulins (HIG) have been used for detection of inflammation. Autologous immunoglobulins have been successfully used for detection of inflammation in animals.

Aim of our study was to assess feasibility of directly labeled autologous IgG, separated from patients' sera, for imaging of infection.

Methods: autologous IgG were separated from patients' sera using fast protein liquid chromatography (FPLC) and directly radiolabeled with ^{99m}Tc. Planar scintigraphy was performed in 18 patients with suspected inflammation.

Results: sensitivity of autologous IgG scintigraphy was 66 % and specificity 80% when compared with other investigations and final diagnosis.

Conclusion: The method seems promising for detection of inflammation although studies comparing radiolabeled autologous IgG compared to heterologous are necessary to prove superiority of either method.

Key words: autologous IgG, Tc-labeled scintigraphy of inflammation – radionuclide imaging; IgG – diagnostic use; technetium – diagnostic use

Introduction

Radiolabeled heterologous polyclonal human gamma globulins (HIG) have been used several years for detection of inflammation[1-4] and accuracy to detect infection is excellent in selected patients.[5] Uptake of IgG in some tumors was shown as well.[2] HIG are easy to prepare and thus useful for routine clinical work although radiolabeled leukocytes are superior in the diagnosis of focal purulent disease.[6] Distribution of various HIG preparations in vivo does not always follow identical pattern, as shown in baboon experiments. Louw et al. believe that it depends on degree of damage of commercially available IgG caused during the preparation.[7]

Dormehl and coworkers[8] showed foci of inflammation with autologous labeled IgG in baboon experiments. They consider autologous IgG most closely related to intact IgG. Labeled autologous IgG are even less likely to cause allergic reaction in recipient than the heterologous ones and could therefore be useful for repeated investigations in humans.

Aim of our study was to evaluate the feasibility and possible contribution of autologous IgG, separated with fast protein liquid chromatography (FPLC) and directly labeled with ^{99m}Tc, for scintigraphy in patients with inflammation.

Correspondence to: Prof. Nataša V. Budihna, M.D., Ph.D., Department of nuclear medicine, University medical center, Zaloška 7, 1000 Ljubljana, Slovenia. Phone: +386 61 316 855. Fax: +386 61 132 72 72. The work was in part supported by research grant no. J3-6208-312-94, Ministry of Science and Technology, Slovenia.

UDC: 616-002.182-07-097

Patients and methods

Separation of Ig G

Five to 10 ml of patient's blood were withdrawn and serum was separated. Serum was exposed to

0.3 % sodium cholate (Merck) for 24 hours before further manipulation to prevent contamination of the chromatographic system.[9]

For separation *of Ig* from the serum, 3.0 ml of serum were precipitated with 3.0 ml of cold saturated ammonium sulfate solution. The resulting suspension was incubated and mixed during next 30 minutes. Centrifugation at 2000 g and 4° C for 15 minutes followed. The supernatant was discarded and the precipitate redissolved in 1.0 ml of 0.15 Mol/L and pH 6.5 phosphate buffer. The resulting solution was precipitated with 1.0 ml of saturated ammonium sulfate and centrifuged under the same conditions again. The precipitate was redissolved in 1.0 ml of phosphate buffer and passed through a 0.22 μm Millipore filter.

Second step of the separation procedure was *IgG* separation. The method was originally used for IgM isolation from human serum.[10] Our modification is described in detail elsewhere.[11] In short, slower flow rate than the originally proposed resulted in improved separation of IgG from IgA. For this purpose, FPLC using strong anion exchange column Mono Q HR 5/5 (Pharmacia), HPLC Gradient Pump 2249 (LKB), ultraviolet light (UV) detector Uvicord SD 2510 (LKB), Rheodyne injector, fraction collector RediFrac (Pharmacia) and recorder REC-481 (Pharmacia) were used.

Sample of 200 ml autologous Ig solution was used with FPLC. Following solutions were used: 0.15 Mol/l phosphate buffer pH 6.5 (buffer A) and 0.30 Mol/l phosphate buffer pH 6.5 (buffer B). Mono Q HR 5/5 was rinsed with 20 % ethanol and equilibrated with buffer A before use. First elution was performed with buffer A for 10 minutes, the second elution with buffer B for 15 minutes at flow rate of 0.5 ml/min.

Different immunoglobulin classes were detected with measurement of UV absorption and separately eluted. IgG were detected at UV wavelength 277 nm and at absorbancy range 1.0 AUFS (absorbance units full scale, as defined by manufacturer). IgG were eluted at the first, IgA at the second and Ig M at the third UV absorption peak.

Confirmation of IgG was accomplished with immunoelectrophoresis. The first fraction of the eluate, containing IgG, was collected separately and its total volume of approximately 0.4 ml was used for direct radiolabeling.

Direct labeling of IgG with ^{99m}Tc

The labeling was accomplished using the method of Pettit[12] with stannous tartrate as reducing agent for pertechnetate. IgG solution was preincubated for 20 minutes at room temperature with 50 ml of 2.1×10^{-3} Mol/L stannous tartrate solution (Sigma) and 500-800 MBq $^{99m}TcO_4^-$ were added in a volume as small as possible. Incubation at 40° C for 10 minutes followed. 100 μl of saturated $NaHCO_3$ were added thereafter and incubated again at 40° C for 20 minutes. The solution was passed through a 0.22 μm Millipore filter.

Quality control

The quality control of radiolabeled IgG was performed in two systems of ascending instant thin-layer chromatography (ITLC) using silica gel strips (Gelman) of 1 x 12 cm size. Methanol (85 %) was used as solvent in the first ITLC system. Free pertechnetate moved within the solvent front, while the radiolabeled IgG and colloidal forms of technetium remained at the start.

Silica gel strips were soaked with serum albumin (5 g/L), rinsed in deionized water and allowed to dry. They were used in the second ITLC system as a supporting phase while the mixture of solvents (C_2H_5OH: NH_4OH: H_2O in 2:1:5 ratio) served as a mobile phase. The colloidal forms of technetium (hydrolyzed or reduced technetium) remained at the start, while the labeled IgG and free pertechnetate moved with the solvent's front.

The percentage of free pertechnetate was calculated from the radioactivity of the solvent's front in the first ITLC system. The radioactivity at the start of the second ITLC system represented the colloidal forms of technetium.

The difference between distribution of radioactivity in the first and in the second ITLC system gave the estimation of radioactivity bound to IgG.[13]

Radiolabeled IgG was incubated in autologous serum at 37° C for 18 hours to test in vitro stability of radiolabeled autologous IgG.

Patients

The study protocol was approved by the national ethical committee. All patients gave informed consent before entering the study.

Eighteen nonselected patients with proven or suspected infection were included. Blood sample was withdrawn one day before the scintigraphic investigation.

Autologous immunoglobulins were labeled with technetium-99m (500 - 800 MBq) on the following day and reinjected intravenously. Acquisition of scintigraphic data followed.

Scintigraphy

Planar spot view scintigrams of the whole body were acquired in anterior and posterior projections with a large field of view gamma camera (Siemens Basicam), equipped with LEAP collimator and connected to a computer (McIntosh IIfx, McLear software for nuclear imaging), at 30 minutes, 3-6 hours and 17 hours after intravenous injection of autologous radiolabeled IgG.

Data analysis

Scintigrams were analyzed by two experienced observers, blinded to the clinical data. The lesion to background uptake of ^{99m}Tc IgG was evaluated visually and the uptake intensities compared. Accumulation of labeled IgG in liver, kidneys, spleen and bone marrow as well as in the blood pool in the first phase after application was considered normal. The uptake in organs and tissues higher than the normal background was considered abnormal. Studies with nonconclusive accumulation in organs other than the normal distribution were reported as equivocal. Final decision was made by consensus in case of disagreement of separate readings of the two observers.

The results of scintigraphy with ^{99m}Tc IgG were compared with final diagnosis and with findings of all available investigations as bone scan, radiograms, computed tomography, ultrasound, erythrocyte sedimentation rate and white blood cell count.

Scintigraphy was validated using sensitivity and specificity of the new method compared with the final diagnosis for all studies. False positive or negative results were analyzed in detail.

Results

IgG separation and labeling

IgG were completely separated from other immunoglobulins in all sera as confirmed with chromatography. The labeling efficiency of isolated autologous IgG was above 95 %. Labeled ^{99m}Tc-IgG were stable in vitro. Only 2.5 % of free pertechnetate and 4.6 % of colloid was detected after 18 hours' incubation at 37 °C.

Patients

No adverse reaction was observed after intravenous application of autologous ^{99m}Tc-IgG to the patients. Seven men and 11 women, in average 46 (±15.6) years old, all with suspected inflammatory lesions were included. Two patients had spondylodiscitis, single patients had discitis and paravertebral abscess, discitis and psoas abscess, paravertebral, suspected pancreatic, gluteofemoral and paranephric abscess. Osteomyelitis was suspected in four patients, pulmonary sarcoidosis in 2 and 4 patients were investigated for suspected myocarditis (Table 1). Average duration of inflammation in patients (except in patients with sarcoidosis and myocarditis) was 2.8 (± 3.1) months. Most patients were treated with antibiotics at the time of scintigraphy. Only 2 patients with suspected osteomyelitis, 2 with sarcoidosis and 3 with myocarditis did not have antibiotic therapy.

Eight suspected inflammatory lesions were true positive and 4 true negative. In one patient with suspected inflammation in recent bone fracture the lesion was false positive. False negative were 3 lesions in patients with psoas or paravertebral abscesses and in one patient with spondylodiscitis in previously irradiated area. Results of scintigraphy in two patients with dilatative cardiomyopathy after myocarditis and in one patient with sarciodosis were equivocal.

Eight of 17 lessions in suspected inflammation were true positive, 4 were true negative, 4 were false positive and 1 false negative; calculated sensitivity of our method (without the three equivocal cases) in detection of inflammation is 66 % and specificity 80 %.

Discussion

Detection of inflammation with heterologous polyclonal human gamma globulins (HIG) is well accepted. As autologous IgG are considered to be most closely related to the intact IgG,[8] they might also have more specific affinity for patient's antigens than the heterologous ones and could therefore be suitable for investigations in humans. Radiolabeled autologous IgG are not likely to cause allergic reaction in the recipient than heterologous polyclonal HIG.

Autologous technetium labeled IgG could be therefore useful for detection and follow-up of patients with inflammation or tumors without risk of immunologic reactions and with low radiation exposure.

Our study was planned to evaluate feasibility of autologous IgG, separated with fast protein liquid

Table 1. Patient's data.

Patient. Number	Gender	Age	Therapy	Final Diagnosis	Disease Duration	Radiogram Computed Tomography	Ultrasound	Cytology	Autologous 99m Tc-IgG Scintigram	^{99m}TC-HMPAO Labeled Leucocyte	Bone Scintigram	Laboratory Tests
1	Female	34		Discitis L4/5	2 months	Discitis			Uptake	Uptake L4/5	ESR 22. L4/5	Leucocytes 8.5
2	Female	35	Anti-biotics	Paravertebral abscess. disciti	1 week	Paravertebral lesion. discitis L4/5			Uptake L4/5		Uptake L4/6	ESR 102. Leucocytes 9.1
3	Female	58	Anti-biotics	Osteosynthesis	2 months	Fragments of bone without callous			Uptake in fracture	Negative	Uptake in fracture	ESR 45. Leucocytes 8.1
4	Female	65	Anti-biotics	Spondylo discitis	10 days	L3/5 degenerative changes			Negative		Low uptake postirradition	EST 120
5	Female	59	Anti-biotics	Paranephritic abscess	2 months		Parane-phric fluid	Pus	Paranephric uptake			ESR 32
6	Female	36	Anti-biotics-	Post osteo-myelitis	5 months				Negative			
7	Female	71	Anti-biotics	Discitis. psoas abscess	6 months	Inflammation left SIS. L4/5. psoas abscess (CT)		Pus in psoas	Uptake L4/5	Negative	Uptake L4/5	ESR 60
8	Female	58	Anti-biotics	Osteomyeitis in femoral fracture	3 months	Partially healed fracture			Uptake femur		Uptake femur	Hemoculture Streptococcus viridans
9	Male	64	Anti-biotics	Gluteofemoral abscess	1 month	Discitis L4/5. pus in thigh			Uptake L4/5. gluteal	Uptake gluteal	Uptake L4/5	Positive hemoculture
10	Male	56		Pancreatic cyst	2 months	Cyst in pancreas	Cyst in pancreas		Negative	Negative		
11	Male	49	Anti-biotics	Paravertebral abscess	3 weeks	Spondylo-lysis L5	Heart. abdomen normal		Negative			Hemoculture Staphyloccocus aureus
12	Male	45		Dilatative myocardiopathy post myocarditis	2 months	Enlarged heart	No pericardial effusion	Few signs of myocar-ditis	Equivocal in myocardium	Antimyosin antibodies positive		
13	Female	20		Postpartal myocardiopathy	1 month	Enlarged heart	Intracardial thrombus		Uptake in the heart			
14	Male	25		Dilative myocardiopathy post myocarditis	18 months	Enlarged heart	No pericardial effusion	Few signs of myocar-ditis	Equivocal in myocardium	Antimyosin antibodies equivocal		
15	Male	74		Pulmonary sarcoidosis	120 months	Thorax radiogram normal			Equivocal			ESR 4
16	Female	44		Pulmonary sarcoidosis	24 months	Hilar lymph nodes			Equivocal			
17	Female	43	Anti-biotics	Sepsis. suspected myocarditis	1 month				Negative			Leucocytes 13.8
18	Male	37		Osteoarthritis	12 months	Osteoarthritis			Negative		Osteoarthritis	

L4/L5 or = fourth to fifth lumbar vertebrae. SIS = sacroiliac joint. CT = computed tomography. L = left. ESR = erythrocyte sedimentation rate (mm/h). Leukocytes = number x 10^9/L. normal 4.0–10.0. ^{99m}Tc-HMPAO = scan with ^{99m}Tc-HMPAO labeled leucocytes.

chromatography (FPLC) and directly labeled with ^{99m}Tc, for scintigraphy of inflammation. Modified flow rate with FPLC allowed complete separation of IgG from other serum proteins. In vivo instability of technetium labeled IgG was shown to be a problem with direct labeling via stannous ion reduction.[14] Hnatowich and coworkers found evidence of in vivo transchelation of ^{99m}Tc to cistein as a cause of high renal radioactivity. They also observed IgG fragmentation after stannous ion reduction. Fragments can be excreted via kidneys as well. Our studies in vivo[11] demonstrated high radioactivity in kidneys and in urinary bladder but not in the thyroid, what is consistent with stable binding of technetium. Stability of directly labeled ^{99m}Tc-IgG complex was in our study proved in vitro. Concentration of radioactivity in lesions was high enough to allow scintigraphic visualization of most inflammatory lesions in spite of shortcomings of direct labeling of autologous IgG with ^{99m}Tc.

The mechanism of IgG accumulation in inflammatory and neoplastic lesions is not entirely understood. One of the most important reasons for IgG uptake in inflammatory lesions is increased vascular permeability and the diffusion at the site of inflammation.[15] Some authors suppose that the attraction between Fc region of IgG and Fc receptors on the leukocytes and bacteria allows accumulation of labeled substances.[16–18] The type of immunoglobulin was shown to play a role in accumulation at the site of inflammation.[19]

The results of scintigraphy in our patients with infection were comparable to the results of studies with heterologous technetium labeled IgG.[4–20] Autologous ^{99m}Tc-IgG are not equally successful in detection of all types of inflammation. Satisfactory results were achieved in patients with spondylodiscitis (Figure 1). The fact that labeled leucocytes have limitations for scintigraphy of inflammation in spinal region[5, 12] warrants further study of this specific application of radiolabeled ^{99m}Tc-IgG. On the other hand, because of high blood pool activities the results in patients with myocarditis and sarcoidosis were equivocal. False negative autologous ^{99m}Tc-IgG scintigram in paravertebral or psoas abscesses was the rule in our study. It was probably due to high background of the spine, liver and kidneys in comparison with the uptake in the abscess. The false negative scan in spondylodiscitis in a patient with previously irradiated spine was probably due to low bone metabolism and lowered perfusion of that area. False positive uptake in a recent unhealed fracture and in some osteoarthritic joints showed the low specificity of the new method. The results of our study are encouraging in patients with spondylodiscitis. Larger group of patients should be studied for more reliable evaluation of sensitivity and specificity of scintigraphy with autologous ^{99m}Tc-IgG. Furthermore, tomographic techniques would improve diagnostic accuracy of our method. Studies comparing technetium labeled autologous with heterologous IgG are necessary to prove possible differences between the two methods. Newer direct labeling techniques[22] probably have advantages for scintigraphy with autologous and heterologous polyclonal IgG.

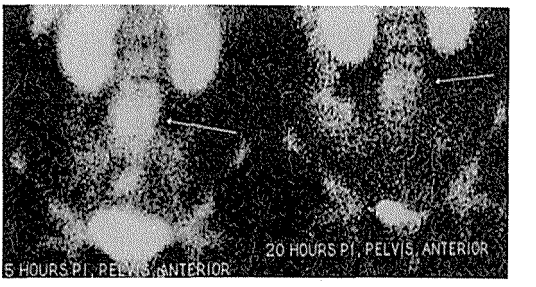

Figure 1. Scintigram with autologous IgG labeled with technetium-99m in a patient with discitis in the lumbar spine. High uptake of technetium-99m labeled autologous IgG is shown in 4th and 5th lumbar vertebrae.

Conclusions

The present study proves safe and feasible use of autologous IgG, directly labeled with technetium, for scintigraphy in patients. Convincing uptake of autologous ^{99m}Tc-IgG was shown in inflammatory lesions like spondylodiscitis. It is suitable for repeated use because there is no danger of allergic reaction in the recipient. The method of separation and labeling is to complicated to be used unselectively in routine clinical practice.

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