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A PROSPECTIVE COMPARISON OF 99mTc-LABELED POLYCLONAL HUMAN IMMUNOGLOBULIN AND 111In GRANULOCYTES FOR LOCALIZATION OF INFLAMMATORY BOWEL DISEASE


Abstract

There is a need for an easily prepared radiopharmaceutical agent for the detection of inflammation and infection. In a group of 14 patients with inflammatory bowel disease (IBD), the detection of actively involved intestinal segments by nonspecific human polyclonal immunoglobulin (IgG) labeled with 99mTc was compared with that of 111In granulocytes. To determine the specificity of 99mTc-IgG scintigraphy, 8 control patients without clinical indications of intestinal inflammation were examined. 99mTc-IgG was found in the left colon in 8 and in the right colon in 7 of the 8 controls 4 hours after the injection. At that time of scintigraphy only 4 IBD patients exhibited a more intense accumulation at the site of the intestinal segments with active disease. In contrast, in a randomized comparison with 111In granulocytes scintigraphy was positive in 11 patients with the latter technique. Moreover, fewer diseased segments were seen in the 4 patients with positive 99mTc-IgG scintigraphy (6 versus 12 with 111In granulocytes). In view of the low sensitivity and specificity, it is concluded that 99mTc-IgG is not suitable for the scintigraphic staging of IBD patients.

Key words: Intestines, infection; --, radionuclide studies; radionuclides, comparative studies.

Scintigraphy with autologous leukocytes labeled with 111In is a very successful approach to the noninvasive diagnosis of abscesses. In gastroenterology, this technique is also applied to establish the localization and severity of inflammation of the intestine in patients with inflammatory bowel disease (IBD) (14–17). For these patients, it appears to be an especially reliable diagnostic modality if the abnormality is located in the colon (6). Disadvantages of 111In leukocytes are: the time-consuming and laborious cell separation and labeling technique; the relatively unfavorable radiation characteristics of 111In which result in only moderate resolution, long acquisition times, and a relatively high radiation load for the patient; and the fact that the relatively expensive radionuclide is not always available. As a result there is a clear need for a better radiopharmaceutical agent that is always "on the shelf" in a kit, preferably one that can be labeled with 99mTc. But most importantly, it must be at least as accurate as 111In leukocytes. In previous studies, oral 99mTc sucralfate and intravenous 99mTc nanocolloid were tested in IBD patients (2, 7, 9, 12, 19, 20). In our department, neither radiopharmaceutical agent was satisfactory in identifying active IBD (2, 7).

Recently nonspecific human polyclonal IgG was introduced for the detection of infection (13). Encouraging results were also obtained for IBD patients with active disease (10, 16). In our hospital good results were obtained in a pilot study of 99mTc-labeled IgG for the mapping of active arthritis in patients with rheumatoid arthritis (RA) (11).

Here we report on a study in which 99mTc-IgG was compared in random order with 111In granulocytes in a group of IBD patients. The aim of the study was to determine whether 99mTc-IgG could replace the technique used until now (i.e. 111In granulocytes) to establish the localization and severity of inflammation in IBD patients when clinical exacerbation of the disease is present.

Material and Methods

Patients. This prospective, randomized, comparative study involved 14 patients (9 males and 5 females) who had been referred for 111In granulocyte scintigraphy for

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The cell pellet, containing erythrocytes and granulocytes, was treated with buffered ammonium chloride at 0°C. After addition of 350 MBq freshly eluted 99mTc pertechnetate, the contents were mixed by gently whirling and then incubated for 15 min at room temperature. The labeled cells were washed twice with 50% autologous plasma in Hank's balanced saline solution and resuspended in 4 ml pure plasma. Depending on labeling efficiency, doses of 2.7 to 4.7 MBq were injected into the patient.

To determine the normal distribution of 99mTc-IgG in the abdomen 4 hours after the injection, we studied 8 control patients believed to be free of intestinal disease, who underwent 99mTc-IgG scintigraphy only for assessment of their RA.

All patients gave informed consent, and the study was approved by the Ethics Committee of the University Hospital, Leiden.

Radiopharmaceuticals. 99mTc-IgG was prepared from a kit (Technescan-HIG, Mallinkrodt Diagnostica, Petten, The Netherlands) according to the instructions of the manufacturer. For each patient a single vial, containing 1 mg of 2-iminothiolane-derivatized polyclonal human IgG and stannous tartrate, was used. The Technescan-HIG consisted of more than 90% IgG and was HIV- and HBsAg-negative. After addition of 350 MBq freshly eluted 99mTc pertechnetate, the contents were mixed by gently whirling and then incubated for 20 min at room temperature. A dose of 350 MBq was drawn from the vial and injected i.v. into the patient.

Autologous granulocytes were separated and labeled with 111In tropolonate as described previously (6). In short, mononuclear cells were separated from 50 ml heparinized blood by Ficoll-Hypaque density gradient centrifugation. The cell pellet, containing erythrocytes and granulocytes, was treated with buffered ammonium chloride at 0°C to lyse the erythrocytes. Subsequently, 50 million granulocytes were labeled with approximately 120 kBq/million cells 111In tropolonate (Mallinkrodt Diagnostica) by incubation for 15 min at room temperature. The labeled cells were washed twice with 50% autologous plasma in Hank’s balanced saline solution and resuspended in 4 ml pure plasma. Depending on labeling efficiency, doses of 2.7 to 4.7 MBq were injected into the patient.

Imaging. In the IBD patients anterior views of the abdomen were acquired 2, 4, 6, and 24 hours after injection of 99mTc-IgG. Total body scans were made 2 and 6 hours after injection. In the control patients anterior views of the abdomen were obtained at 4 hours only. All images were taken with a rectangular LFOV gamma camera (Toshiba 90B, Toshiba) equipped with a LEAP collimator. The peak energy setting was 140 keV, the peak photon energy of 99mTc. Imaging time for the spot films was 5 min up to 6 hours after the injection and 300,000 counts for the image at 24 hours. The total body images were taken in the whole body mode with a scan speed of 30 cm/min. An anterior view of the abdomen was taken with an LFOV gamma camera (Toshiba 501) 3 to 4 hours after injection of the autologous 111In granulocytes. The camera was equipped with a medium-energy collimator; the peak energy settings were 171 and 246 keV, the peak photon energies of 111In with 2 windows of 20%. Since the injected dose of 111In ranged between 2.7 and 4.77 MBq, the imaging time was at least 20 min.

Interpretation. The 99mTc-IgG scintigrams were printed on transparent film. Two observers read all 99mTc-IgG scintigrams. The readers were not aware of the clinical indications or the results of other radiologic techniques. Six segments of the colon and 2 regions equivalent to small bowel localization were scored on the basis of comparison of the visible

<table>
<thead>
<tr>
<th>Pat No. Age/Sex</th>
<th>Disease</th>
<th>Interval Granulocyte-IgG (days)*</th>
<th>Localization 111In-granulocyte</th>
<th>Localization 99mTc-IgG</th>
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* n: In-granulocyte scan performed n days before IgG-scan.
Fig. 1. Example of anterior abdominal image obtained 4 hours after injection of $^{99m}$Tc-IgG in a patient with RA believed to be free of intestinal inflammation (control subject). There is activity of at least grade 2 in the left colon and midlower abdomen, and grade 1 in the right-sided colon. Images like this one are interpreted as normal.

radioactivity with that of the bone marrow of the iliac crest: 0 = no activity; 1 = slight activity, less than bone marrow; 2 = activity equal in intensity to that of bone marrow; 3 = activity greater than bone marrow; and 4 = much greater than bone marrow. First the 2 observers scored the 4-hour scintigrams only of the IBD patients and the 8 control patients blindly according to this system. Then the complete examination of each IBD patient, i.e. the 2, 4, 6, and 24-hour scans, were read. The $^{111}$In-granulocyte scintigrams were interpreted by another experienced observer, who was only informed about surgical resections previously performed.

Since the purpose of the study was to determine whether $^{99m}$Tc-IgG could replace $^{111}$In-granulocytes for noninvasive visualization of the localization and severity of inflammation, the results of the 2 methods were first compared directly. Endoscopic reports, radiodiagnostic findings, if available, and in one case surgical outcome served as controls of the results.

Results

$^{99m}$Tc-IgG scintigrams revealed intense radioactivity in the kidneys, liver, and bladder over 24 hours. Less intensive storage occurred in the spleen. Moreover, 24 hours after the injection, intravascular radioactivity was also clearly visible, as indicated by the fact that the cavities of the heart as well as the large blood vessels (such as the aorta, vena cava, and iliac veins) became and remained visible. Radioactivity was also visualized in the bone marrow of the pelvis and spinal column.

On the $^{99m}$Tc-IgG scintigrams of the 8 control patients taken 4 hours after the injection (Fig. 1) radioactivity could be seen in the region of the descending colon: score 1 in one case, score 2 in the other 7 cases. In the region of the cecum and ascending colon, activity was less intense: score 0 in one, score 1 in 4, and score 2 in 3 cases. In one case score 3 was given to the transverse colon. When the 4-hour scintigrams of all 22 patients (controls and IBDs) had been read by the 2 observers, it appeared that there were 5 scintigrams showing one or more accumulations of $^{99m}$Tc-IgG that received a score of 3 or 4. One of these 5 cases was the control patient mentioned above (score 3 for the transverse colon), the other 4 had IBD (patients 3–5, 13).
When the 4 scans (2, 4, 6, and 24 hours after injection) for each of the 14 IBD patients were scored by the 2 observers, 4 scintigrams showed an accumulation of $^{99m}$Tc-IgG during the first 4 hours high enough to be given a score of 3 or 4 (patients 3–5, 13). A total of 6 segments was involved. On the scans taken at 6 and 24 hours, diffuse activity throughout the entire colon, increasing in intensity, was observed in all cases.

On the $^{111}$In-granulocyte scintigrams, no pathologic accumulation of the labeled granulocytes was seen in 3 cases. One of these patients (No. 10) was found at surgery to have an infiltrate surrounding the neoterminal ileum. In the 2 other cases (Nos 7 and 12), endoscopic examination 8 and 27 days, respectively, beforehand had revealed a moderately severe sigmoiditis and pancolitis. For the 11 remaining patients, the pathologic accumulation of the $^{111}$In-granulocytes was compatible with active IBD and agreed in localization with the findings of other imaging techniques (Table).

When the findings of the $^{99m}$Tc-IgG scintigrams taken 4 hours after injection are compared with the $^{111}$In-granulocyte examinations, it appears that the accumulations of $^{99m}$Tc-IgG with a score of 3 or 4 in 4 IBD patients were localized in 6 intestinal segments that were also pathologic on the $^{111}$In-granulocyte scintigrams (Table, Figs 2, 3). However, as can be seen in the Table, the $^{111}$In-granulocyte examinations of these 4 patients revealed a total of 12 pathologic segments (Figs 2, 3). For 7 other patients, the $^{111}$In-granulocyte scintigrams showed abnormalities that did not receive a score of 3 or 4 on the $^{99m}$Tc-IgG scans. The opposite situation (i.e. $^{99m}$Tc-IgG score 3 or 4, $^{111}$In-granulocyte scan negative) did not occur.

Discussion

The results of this study show that $^{99m}$Tc-IgG is not suitable for the scintigraphic staging of IBD patients.

$^{111}$In-granulocytes have proved to be reliable for assessment of active IBD. This is probably due to the low background activity obtained in the abdomen, outside liver and spleen. The lack of excretion via the normal digestive tract makes it possible to determine the severity of the IBD by means of the excretion of $^{111}$In in feces (8). Since this implies multiple doses of the $^{111}$In-granulocytes to one patient, low doses of $^{111}$In are given because of the radiation burden. As we have reported previously, these low doses do not yield optimum visualization (1). Despite the fact that some $^{111}$In-granulocyte scintigrams of the patients studied were of lower quality and led in 3 cases to possible false-negative interpretations, the $^{111}$In-granulocytes were considerably more suitable for demonstrating segments with active disease than $^{99m}$Tc-IgG.

Due to the apparently slow blood clearance of $^{99m}$Tc-IgG, accumulation in kidneys, bladder and liver, and excretion into the normal gastrointestinal tract, the background radioactivity in the abdomen remains high. In order to be able to diagnose a focus at the site of an active inflammation, therefore, a very high concentration is required. This could occur if there was a specific binding site for the IgG, as has been demonstrated for some bacterial infections (5). Hyperemia and leakage to the inflamed area apparently cause insufficient concentration. Leakage to and dilution in the lumen of the intestine after eventual accumulation do not lead to a high target-to-background ratio at the site of the inflammation. The apparently physiologic excretion (breakdown products) of this tracer into the intestine will also lead to false-positive interpretations. We could only avoid this by defining the pathologic regions as those with a score of 3 or more and by not using scans taken at 6 and 24 hours. If score 2 had been considered pathologic, then 7 of the 8 RA patients would have had left-sided colitis, and 4 of the 8 a right-sided colitis as well. In fact, in RA patients, NSAID medication can cause a mild inflammation of the small and large bowel which can lead to the loss of blood and proteins. If the $^{99m}$Tc-IgG were also to leak during such an inflammation in the small bowel, then predominant accumulation would have been seen in the ileocecal region (3). However, the highest activity was registered in the left colon, as well in the controls as in the majority of the IBD patients. Therefore, we assume that the $^{99m}$Tc activity visible in the intestine is attributable to physiologic excretion of breakdown products of $^{99m}$Tc-IgG into the large bowel and is concentrated in the left-sided colon lumen as water is reabsorbed and propulsion of the feces is diminished. As we saw no accumulation in the thyroid or the stomach wall at any stage of the examination, free $^{99m}$Tc-pertechnetate is not likely to be the end product of the breakdown. For ethical reasons we did not feel it was justified to subject control patients to $^{111}$In-granulocyte scintigraphy as well or to examine completely healthy volunteers with $^{99m}$Tc-IgG.

The results of this study oppose those of BUSCOMBE et al. (4) who compared $^{99m}$Tc-IgG with $^{111}$In-granulocytes in 16 patients with IBD and found 6 concordant positive and 10 concordant negative results. In their report, they did not mention colon activity that was not caused by inflammation. Our findings are, however, in agreement with those of SPINELLI et al. (18) who were also unable to visualize clear accumulation of $^{99m}$Tc-IgG at the site of diseased intestinal segments; they also refer to activity in the colon that was not attributable to the inflammations.

The poor results of $^{99m}$Tc-IgG scintigraphy for IBD patients are at variance with the good results obtained with this radiopharmaceutical agent for patients with active RA (11).

Conclusion. With $^{99m}$Tc-IgG it was not possible to demonstrate diseased intestinal segments with high sensitivity in the group of 14 patients with active IBD studied. Only in a small number of cases was a portion of the involved intestinal segments correctly diagnosed. This was due especially to the fact that the background activity remains high; in addition, however, there also appeared to be physiologic activity in the colon. In our opinion, therefore, $^{99m}$Tc-IgG is not suitable for the demonstration of the location and
severity of an active inflammation in patients with IBD. For the time being, $^{111}$In-granulocytes are best suited for this purpose.

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REFERENCES


