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A N I N T E R N A T I O N A L J O U R N A L

Hank's Balanced Salt Solution: an Alternative Resuspension Medium to Label Autologous Leukocytes. Experience in Inflammatory Bowel Disease

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ABSTRACT

In this work Hank's balanced salt solution (HBSS) has been used, as resuspension medium, instead of leukocyte poor plasma (LPP) to label autologous white blood cells in 28 patients with suspicion af active inflammatory bowel disease.Labelled cells were reinjected and anterior and caudo-craneal views were obtained at 30 min, 2 h and 6 h p.i. Regions of interest were outlined on liver, spleen, lung, bone marrow (spine), background and lesions and the organ/background activity ratios were calculated in all scans. Patients were classified into 2 groups: Group 1: LPP, 30 patients and Groups 2: HBSS, 28 patients. Labelling efficiency was higher in HBBS group (89.0 ± 3.2 %) than in the LPP group (6.5 ± 6.3%). Organ/background activity ratios were similar in both groups. Concerning diagnostic accuracy was similar at 30 min and 2 h but the false positive rate increased at 6 h p.i. in the HBSS group. HBSS seems to be a valid alternative as resuspension medium in the labeling of autologous leukocytes but leukocyte poor plasma seem to induce less leukocyte damage. Based on these results, in our center HBSS is the currently used medium to label leukocytes.

Key words: ^{99m}Technetium, leukocytes, Hank' s solution, inflammatory

INTRODUCTION

The excellent performance of radiolabelled leukocytes for imaging infection and inflammation has been widely demonstrated. (Peters AM.,1994; Datz et al., 1994; Datz et al.,1986). Recently new radiopharmaceuticals labelled with technetium-99m as cytokines (interleukin- IL-2, IL-6, IL-8); human polyclonal immunoglobulin (HIG); radiolabelled antigranulocyte monoclonal antibodies, radiolabelled peptides and antibiotic have been developed to improve specificity in diagnosis of inflammation and infection (Huub et al., 2001; Mairal et al., 1995; Becker et al., 1994; Vinjamuri et al., 1996; Huub et al., 2001b; Martin-Comin et al., 1999). However, the availability of these new agents is limited and 99mTc-labeled leukocytes scintigraphy (WBC-scan) is looked as the best nuclear medicine available technique for infection and inflammation imaging.

In recent experience leukocyte poor plasma (LPP) has been substituted by Hank's Balanced Salt Solution (HBSS) as resuspension media in the labelling method of autologous leukocytes using 99mTc-HMPAO as labelling agent.

HBSS is a well-known biological medium that is used in different procedures to stabilise and buffer suspended cells. The use of LPP requires blood

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derivatives handling and may be different from one patient to another.
In this work the labelling efficiency, biodistribution and the diagnostic efficacy of leukocytes labelled using HBSS is analysed and compared with those labelled using leukocyte poor plasma.

MATERIAL AND METHODS

Labelling procedure

Fifty-eight consecutive patients have been studied. Autologous leukocytes were labelled with ^{99m}Tc-HMPAO using LPP or HBSS as resuspension medium. The agent was selected by the radiopharmacist without knowledge of the physicians who read the images.

Cell labelling

Leukocyte poor plasma group: 45 mL of venous blood collected over 6 mL acid citrate dextrose were obtained from 30 patients (19 men, 11 women). The leukocyte-rich pellet was obtained from each blood-acid citrate dextrose sample using the hydroxy-ethyl-starch sedimentation technique according to the consensus protocol of the International Society of Radiolabelled Blood Elements (Roca et al., 1998).
The leukocyte-rich pellet was gently resuspended in 0.5 mL cell-free plasma using a polypropylene Pasteur-type pipette. Afterwards, 0.5 mL ^{99m}Tc-exametazime (≈ 280 MBq, freshly prepared) was added. The incubation medium was mixed by swirling the test tube and was incubated for 15 min at 37ºC.
Immediately after incubation, 4 mL cell-free plasma were added to the test tube. Afterward, the tube was centrifuged at 150g for 5 min. The plasma supernatant containing the unbound ^{99m}Tc-exametazime was removed and the ^{99m}Tc-labeled leukocytes pellet suspended in 4.0 mL cell-free plasma.
HBSS group: 45 mL of venous blood collected over 6 mL acid citrate dextrose were obtained from 28 patients (12 men, 16 women). The leukocyte-rich pellet was obtained from each blood-acid citrate dextrose sample using the same technique as LPP group.
The leukocyte-rich pellet was gently resuspended in 4.0 mL HBSS using a polypropylene Pasteur-type pipette and the test tube centrifuged at 150g for 5 min. The plasma-HBSS supernatant was removed and the leukocyte-rich pellet was gently resuspended in 0.5 mL HBSS.
Afterwards, 0.5 mL ^{99m}Tc-exametazime (≈ 280 MBq, freshly prepared) was added. The incubation medium was mixed by swirling the test tube and was incubated for 5 min at 37ºC.
Immediately after incubation, 4 mL HBSS were added to the test tube. Afterward, the tube was centrifuged at 150g for 5 min. The plasma-HBSS supernatant containing the unbound ^{99m}Tc-exametazime was removed and the ^{99m}Tc-labeled leukocytes pellet suspended in 4.0 mL cell-free plasma.

Scintigraphic Imaging

The first scan, early scan, was obtained 20-30 min after 99mTc-HMPAO leukocytes reinjection while the second scan was obtained about 2 hrs post-injection of 99mTc-HMPAO leukocytes, finally a third anterior abdominal scan was obtained at 6 hours p.i. In early and late imaging anterior and caudo-craneal (tail-on the camera) views were obtained.
Regions of interest were outlined in lung, spleen, liver, bone marrow, maximal bowel uptake (when scan showed bowel lesions) and background (central abdominal area free of spine and bowel).

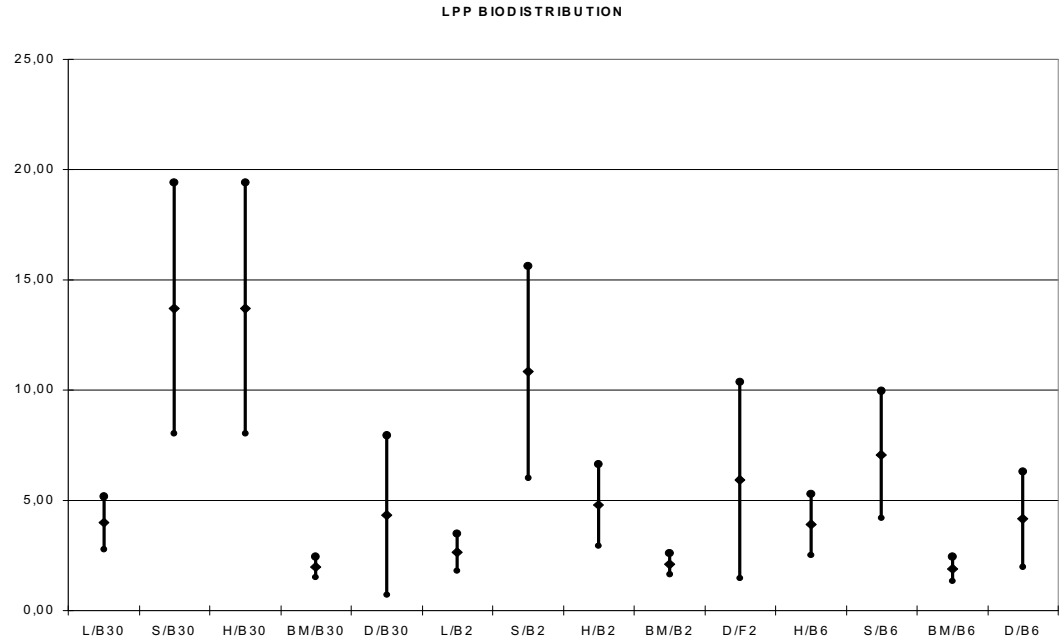
RESULTS AND DISCUSSION

Labelling efficiency:

The leukocytes labelling efficiency with 99mTc obtained in the leukocyte poor plasma group (N=30) ranged from 55,3 % to 75, 4% with a mean value of 65,5 ± 6,3%. In the HBSS method (N=28) the mean value was 89,0 ± 3,2 %, ranging from 82,2% to 95,7%. Differences were statistically significant $p \leq 0,005$.

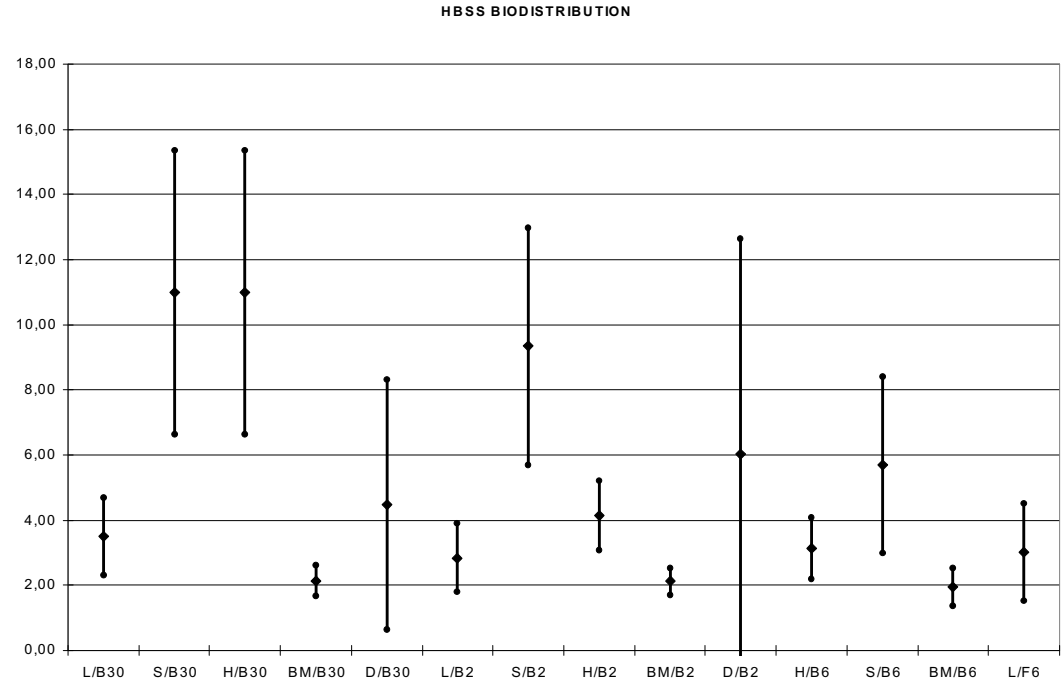
Biodistribution:

Biodistribution was similar in both groups. Thirty minutes p.i. the highest uptake was concentrated in spleen and liver. Liver uptake decreased significantly at 2 hours while spleen uptake decreased softly from 30 min up to 6 h p.i.



The values represent the mean ± sd
(L: lung, B: background, S: spleen, H: hepatic, BM: bone marrow, D: disease segment)

Figure 1 - shows organ/background activity ratios in both groups.



The values represent the mean ± sd
(L: lung, B: background, S: spleen, H: hepatic, BM: bone marrow, D: disease segment)

Figure 2 - shows organ/background activity ratios in both groups.

Lung and bone marrow uptake was significantly lower than spleen and liver one and did not show any change with time. Lesion uptake showed a wide distribution depending on the severity of lesions. Figures 1 and 2 show the organ/background ratio at 30 min, 2 h and 6 h post injection of labelled leukocytes. (L: lung, B: background, S: spleen, H: hepatic, BM: Bone marrow, D: disease segment).

Experience in inflammatory bowel disease:

Table 1 shows the results in the 30 patients of the LPP group and the 28 patients of the HBSS group. The presence of active disease was demonstrated by endoscopy/rectoscopy and biopsy in most patients, by surgery in 4 cases and 1 case only CT data were available. In patients with negative scan for IBD, diagnosis was established by endoscopy.

Table 1 - Results of 99mTc-HMPAO leukocytes imaging of patients with inflammatory bowel disease

Labelling Method	True positive	False positive	True negative	False negative	Total
99mTc-WBC LPP Group	22	0	7	1	30
99mTc-WBC HBSS group	18	0	9	1	28

HBSS: Hanks' Balanced Salt Solution; LPP: Leukocyte poor plasma

In the LPP group active IBD was demonstrated in 23 cases (22 TP and 1 FN). Active disease was confirmed by endoscopy/rectoscopy in 20 cases. In 2 cases the patients underwent surgery, which confirmed Crohn´s disease (CD). In the remaining case, the false negative case, scans did not show any abnormal uptake while rectoscopy indicated the presence of CD. Absence of active disease was confirmed by endoscopy in 7 patients (in one case one ischemic colitis was diagnosed).

In the HBSS group active IBD was demonstrated in 19 cases (18 TP and 1 FN). Active disease was confirmed by endoscopy in 14 cases, by contrast radiology in one case, by CT in one case of pancolitis and by surgery in 2 cases. The remaining case, the false negative case, presented normal scintigrams while endoscopy showed the presence of ulcerative colitis (UC) lesions (response to treatment). On the other hand, absence of active disease was confirmed by endoscopy in 10 patients (6 showed normal endoscopy, 3 cases had diverticulosis, one case ischemic colitis and one case had lymphadenitis.

Table 2 shows the values of sensitivity, specificity, negative predictive value and accuracy for both tracers.

Table 2 - Accuracy of 99mTc-HMPAO WBC Scintigraphy in patients with inflammatory bowel disease

Labelling Method	Sensitivity	Specificity	VPN	Accuracy
99mTc-WBC LPP	95,6%	100%	87,5%	96,6%
99mTc-WBC HBSS	94,7%	100%	90%	96,4%

HBSS: Hanks' Balanced Salt Solution; LPP: Leukocyte poor plasma

In 36 patients disease extension was evaluated at 6 h p.i. and compared with early and late scans. No differences were seen between early (30 min) and late scan (2h); the number of segments detected was 29 and 30 for LPP group and 19 and 22 for HBSS group. Nevertheless at 6 h p.i. the number rise to 38 in both groups (table 3). It is important to remark that 4 cases in the LPP in and in 12 of the HBSS group the number of segments increased being positive the previous scans and more significant, in one case of LPP group and in 4 of the HBSS group, 30 min and 2 h scans were normal while the 6 h scan showed abnormal uptake, which means 5 false positive at this time.

Table 3 - Number of segment with disease found with both agents at 30 min, 2 h and 6 h p.i.

Time p.i.	LPP group	HBSS group
30 min	29	19
2 h	30	22
6 h	38	38

In summary, HBSS seems a good alternative to LPP plasma as resuspension medium to label white blood cells. Labelling efficiency is higher and diagnosis accuracy for IBD is similar.
On the other hand the higher number of false positive cases found at 6 h p.i. may be related to a better preservation of leukocyte when LPP is used. Based on these results in our department HBSS is the first choice method to label leukocytes.

RESUMO

Neste trabalho a solução de Hank's (HBSS) foi usada com meio de marcação de leucócitos autólogos, substituindo o plasma pobre em leucócitos (LPP), em 28 pacientes com suspeita de doença inflamatória intestinal. Estas células marcadas foram reinjetadas e as imagens nas visões anterior e caudo-craneal foram realizadas 30 min, 2 h e 6h após a reinjeção. Regiões de interesse como fígado, baço, pulmão, medula óssea (vertebral), background e lesões e a relação orgão/BKG tiveram a atividade determinada em todas as imagens. Os pacientes foram divididos nos grupos LPP (30) e HBSS com 28. A eficiência de marcação foi mais alta no grupo HBSS (89,0% ± 3,2%) do que no grupo LPP (65,5% ± 6,3%). A relação de atividade orgão/ BKG foi similar em ambos os grupos e também a acurácia diagnóstica em 30 min e 2 hr, mas, a taxa de falsos positivos aumentou em 6 hr no grupo HBSS. O meio HBSS mostrou ser uma alternativa válida na marcação de leucócitos autólogos, mas, o LPP parece induzir menos danos aos leucócitos. Com base nestes resultados, em nosso Centro o HBSS é o método de escolha para marcar leucócitos.

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