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The Labeling Efficiency of Invitro F-18 Fluorodeoxyglucose-Leukocyte

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Abstract
Introduction: In our study, we aimed to investigate the effect of leukocyte incubation temperature, F-18 FDG dose and the use of insulin in the process on leukocyte labeling efficiency.
Methods: The study was conducted in the nuclear medicine department of Pamukkale University Medical Faculty between April and October 2019 and is a prospective study.. Leukocytes were isolated from blood from 24 volunteers (age ranges 25-55 years) with fasting blood glucose of 80-120 mg/dl. Groups were incubated at 37°C or room temperature and labeled with F-18 FDG (2mCi/10 mCi). Each group was divided into two groups as insulin added and not added. Bound and free component activity were measured separately with the dose calibrator.

Results: There was no statistically significant difference between insulin-free and insulin-supplemented group (39.5±0.072%; 40.2±0.073, respectively) (p>0,05). In the insulin free group, the percentage of labeling of high-dose F-18 FDG (10 mCi) was found to be significantly higher than low-dose F-18 FDG (2 mCi) (p=0.049), whereas in the insulin-treated group, there was no significant difference between low-dose and high-dose F-18 FDG addition (p=0.09). It was found that the percentage of labeling was statistically higher in the 37˚C incubated insulin added (p=0.036) and non-insulin added (p=0.042) groups compared to the groups that were incubated at room temperature.

Conclusion: Leukocyte labeled with F-18 FDG is a method that can be used in the diagnosis of infection. High temperature incubation and high dose F-18 FDG marking increases the labeling efficiency, while insulin administration does not alter the labeling efficiency

Key words: F-18 FDG, leukocyte labeling, insülin, infection imaging

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1. Introduction

Determination of the presence and location of infection and inflammation is of primary importance for the initiation and maintenance of correct treatment [1]. Nuclear medicine methods are frequently used to detect the focus of infection. In particular, labeling autologous leukocytes with radioactive substances increases the sensitivity of infection imaging. In-111/Tc-99m-hexamethylpropyleneaminoxime (HMPAO)-labeled leukocyte scintigraphy is considered the standard methods for imaging infection [2,3]. However, due to physiological uptake in the urinary system and intestines, infection focuses in these areas may not be evaluated. In addition, resolution and image quality are not perfect.

F-18 fluorodeoxyglucose position emission tomography (F-18 FDG PET) is mainly used for cancer imaging, but it is also useful for infection/inflammation imaging [4,5]. The advantages of the examination are that the process is completed in a short time and the image resolution is superior. F-18 FDG uptake in the area of infection/inflammation occurs due to increased glycolytic activity in inflammatory cells (particularly in leukocytes and activated macrophages) [6]. However, F-18 FDG is a nonspecific agent. Therefore, new methods have been considered to increase the sensitivity in the diagnosis of infection with F-18 FDG PET. Thus, the idea of injecting autologous leukocytes with F-18 FDG by invitro labeling was created. Osman and Danpure, human leukocytes in vitro by marking F-18 FDG in the first study on this issue [7]. In the literature, although the specificity of F-18 FDG-labeled leukocyte PET/CT is superior to F-18 FDG PET/CT, there are some problems that need to be overcome regarding labeling efficacy [8-10]. In the literature, very different values were found in terms of labeling efficiency and stability [11,12]. Furthermore, the short half-life of the F-18 causes some problems in terms of logistics and marking time.

Increasing the efficiency of in vitro leukocyte labeling with F-18 FDG has recently been studied. The effect of insulin at the molecular level is to activate the GLUT receptors and to increase the entry of glucose into the cell. In the literature, different results have been presented regarding the effect of insulin on leukocyte labeling efficacy with F-18 FDG [13-15]. In addition, there are few studies investigating the effect of different parameters such as incubation temperature and F-18 FDG dose on labeling efficiency [15].

In our study, we aimed to investigate the effect of leukocyte incubation temperature, F-18 FDG dose and the use of insulin in the process on leukocyte labeling efficiency in F-18 FDG labeled leukocyte.

2. Material and methods

The study was conducted in the nuclear medicine department of Pamukkale University Medical Faculty between April and October 2019 and is a prospective study. Twenty-four healthy volunteers (14 males, 10 females; age range 25-55) without any disease were included in the study. Fasting blood glucose was measured in the 80-120 mg/dl range on the day of the procedure. This study obtained the approval of the ethics committee of the Non-Interventional Clinical Research Ethics Committee of School of Medicine (60116787-020/28712). Informed consent forms were signed for all patients.

The study was performed by working in laminar flow under in sterile vitro conditions. Fasting blood glucose levels of the volunteers were measured with glucometer and blood was taken from the patients with a determined value of 80-120 mg/dl. 3,5 cc Acit-Citrat-Dextrose (ACD) as anticoagulant was added in enjectors and 20 ml venous blood was collected. Blood with ACD was poured into incubation tube and 5 cc HESPAN added. The blood sample incubated for 45-60 minutes. Thus, red blood cells were allowed to collapse. The supernatant plasma was taken into a separate tube with a sterile pipette. The separated leukocyte-rich plasma was placed in the centrifuge (Electromac M415P). Leukocyte and platelet-rich plasma separated into tubes were centrifuged at 1380 rpm for 10 minutes. The supernatant separated was removed by pipette. The supernatant was removed by pipetting. 4 cc serum aline (SF) was added to the precipitate to allow the dispersion of the precipitate into the SF and centrifuged at 1480 rpm for 5 minutes. After the supernatant was separated by pipette after centrifugation, a leucocyte-rich pellet was formed at the bottom.

The leukocyte precipitate diluted with SF was divided into 2 separate tubes. Until this stage, the same procedure was applied to each volunteer's blood. In the following steps, 3 different methods were used:

Method 1: Leukocytes prepared in the same way, 10 IU insulin was added to one of the tubes by dividing into two separate tubes and waited for 20 minutes at 37˚C. No insulin was put in the other tube. Then 10 mCi F-18 FDG was added to each tube and incubated at 37°C for a further 15 minutes. 37˚C temperature was provided with Nüve-NB5 type boiler.

Method 2: Isolated leukocytes as described above were divided into 2 separate tubes. 10 IU insulin was added to one of the tubes and allowed to stand at room temperature (25ºC) for 20 minutes. No insulin was put in the other tube. 10 mCi F-18 FDG was added to each tube and left for 15 minutes at room temperature.

Method 3: Isolated leukocytes were divided into 2 separate tubes. 10 IU insulin was added to one of the tubes and allowed to stand at room temperature (25ºC) for 20 minutes. No insulin was put in the other tube. To each tube 2 mCi F-18 FDG was added and left for 15 minutes at room temperature.
In our study, short-acting insulin preparation was used as insulin (NovoRapid Flexpen-Novo Nordisk-Bagsvaerd Denmark). The materials prepared in each 3 tubes were centrifuged at 1650 g for 5 minutes and bound leukocytes were isolated. The precipitate formed at the end of the centrifugation was leukocytes bound by F-18 FDG and the supernatant was the free component. The free component was pulled out with the syringe and separated from the connected component. Bound and free component activity were measured separately with the dose calibrator. The percentages of bound and free components were calculated by dividing the values into total activity.

Statistical analysis
Data were analyzed with SPSS 24.0 (IBM SPSS Statistics 24 software (Armonk, NY: IBM Corp.)). Continuous variables were expressed as mean±standard deviation, median (minimum -maximum values). The suitability of the data for normal distribution was examined by Shapiro-Wilk test. One-way ANOVA was used to analyze the independent group difference. $p<0.05$ was considered statistically significant.

3. Results
The labeled leukocyte activities, total activities and labeling efficiency were classified for the groups we formed according to the variables in Table 1.
In our study, the mean labeling efficiency in the insulin-free group was 39.5±0.072% (range: 23%-52%); the mean labeling efficiency in the insulin-supplemented group was 40.2±0.073% (range: 27%-58%).
When the effect of insulin on F-18 FDG-leukocyte labeling efficiency is examined; the use of insulin did not make a statistically significant difference for the groups using 10 mCi F-18 FDG dose at 37˚C, 2 mCi F-18 FDG dose at 37˚C and 2 mCi F-18 FDG at room temperature. ($p> 0.05$) (Table 2).
The effect of F-18 FDG dose on labeling efficiency percentage is shown in Table 3. In the non-insulin-treated group, the labeling efficiency with high-dose F-18 FDG was significantly higher than those with low-dose F-18 FDG ($p=0.049$). In the insulin group, the addition of low-dose and high-dose F-18 FDG was not statistically significant ($p=0.09$).
The effect of incubation temperature F-18 FDG-leukocyte labeling efficiency is shown in Table 4. It was found that the percentage of binding was statistically higher in the 37˚C incubated insulin added ($p=0.036$) and non-insulin added ($p=0.042$) groups compared to the groups that were incubated at room temperature.

4. Discussion
In our study, the average labeling efficiency in the insulin-free group was 39.5±0.072% (23%-52%); the mean labeling efficiency in the insulin-supplemented group was 40.2±0.073 (27%- 58%). In the literature, there are few studies on F-18 FDG leukocyte labeling and there are significant differences between the binding efficiency values obtained in these studies. In the study of Osman and Danpure, F-18 FDG leukocyte labeling efficiency found ranging from 2% to 80% [7]. In the study, they made labeling by changing the glucose level. They reported that the high variability in labeling efficiency was related to this. In our study, we did not change the glucose level and therefore we did not observe any wide differences between our labeling efficiency values. In their study, Forstrom et al. [15] found that F-18 FDG leukocyte average labeling efficiency was 62.7±1.6%. They showed that 80% of the bound activity was in the granulocyte fraction, 14% was in the mixed lymphocyte-plasma fraction and 6% was in the plasma fraction. Since we considered our study for routine PET/CT practice, we did not divide leukocytes into fractions. Pellegrino et al [8], leukocytes isolated from 20-40 ml of blood with 10-20 mCi F-18 FDG were labeled by incubation at 37˚C for 20 minutes and found the average labeling efficiency in the range of 64-75%. We thought that this difference may be due to the amount of blood used. Therefore, the number of leukocytes that can be marked will be higher. Dumarey et al. included 21 patients and found that F-18 FDG leukocyte mean labeling efficiency was 75±21% (24-96%) [11]. Blood samples of patients diagnosed with infection were used in these studies. We conducted the study with blood samples taken from healthy volunteers aged 25-55 years without any infectious or chronic disease. Considering the increase in the number of leukocytes in the infected blood and the increase of GLUT receptor/hexokinase enzyme activity in these leukocytes, we thought that the difference in the mean labeling efficiency between these studies and our study was dependent on the selected patient profile.

Table-1: Working groups and obtained data

	Insulin-Free Group			**Insulin-Administered Group**		
Grups	Labeled Leukocyte Activity (mCi)	Total Activity (mCi)	Labeled efficiency (%)	Labeled Leukocyte Activity (mCi)	Total Activity (mCi)	Labeled efficiency (%)
37ºC 10 mCi						
1.	4,60	8,84	% 50	3,88	8,64	% 52
2.	3,63	7,89	% 46	2,96	7,40	% 40
3	3,59	7,48	% 48	3,75	7,66	% 49
4.	3,12	6,78	% 46	3,19	7,08	% 45
5.	3,13	8,03	% 39	3,49	7,92	% 44
6.	3,47	8,26	% 42	3,61	8,02	% 45
7.	2,72	6,81	% 40	2,68	7,45	% 36
8.	4,59	8,82	% 52	4,62	7,97	% 58
37ºC 2 mCi						
1.	0,73	1,62	% 45	0,83	1,77	% 47
2.	0,52	1,27	% 41	0,55	1,41	% 39
3.	0,58	1,48	% 39	0,82	1,68	% 40
4.	0,65	1,81	% 36	0,53	1,39	% 38
5.	0,49	1,16	% 42	0,52	1,18	% 44
6.	0,37	1,08	% 34	0,39	1,14	% 34
7	0,60	1,34	% 45	0,58	1,32	% 44
8.	0,39	1,02	% 38	0,52	1,27	% 41
25 ºC 2mCi						
1.	0,34	1,49	% 23	0,32	1,18	% 27
2.	0,71	1,74	% 41	0,72	1,80	% 40
3.	0,46	1,63	% 28	0,41	1,32	% 31
4.	0,34	1,30	% 26	0,44	1,47	% 30
5.	0,41	1,17	% 35	0,40	1,26	% 32
6.	0,74	1,86	% 40	0,55	1,58	% 35
7.	0,60	1,57	% 38	0,68	1,73	% 39
8.	0,55	1,66	% 33	0,38	1,08	% 35

In our study, we did not observe a statistically significant difference in F-18 FDG leukocyte labeling efficiency between the insulin and non-insulin groups. In one study, they examined the effect of insulin incubation with F-18 FDG on leukocyte labeling, and found that insulin had no effect on lymphocyte labeling ($p < 0.05$), and that monocytes increased significantly ($p < 0.01$) [13]. In this study, they performed the labeling with 10 mCi F-18 FDG at 37˚C and incubated with 10-100 IU insulin for 3 hours before labeling and 30 min after the addition of F-18 FDG. Since we did a study of the routine practice of leukocyte labeling with F-18 FDG, we did not fractionate leukocytes and tested overall labeling efficacy. We used fixed-dose short-acting insulin in the groups to which insulin was added and incubated for 20 minutes after F-18 FDG addition. Fostrom et al, 100 mU regular insulin used in their study and

they found that insulin has no significant effect on F-18 FDG leukocyte labeling efficiency [15]. Moon et al. [14] found 40.3±6% labeling efficiency in the insulin-free group and 40.3±7.7% in the insulin group. They reported no statistically significant difference in these results. These results are consistent with the results obtained in our study.

Table-2: The effect of insulin on F-18 FDG leukocyte labeling efficiency

Grups	Mean (±SD) (%)	Median(min-maks.) (%)	P value
Without insulin-2 mCi-37˚C	40,0 (±0,040)	40,0 (0,34–0,45)	0,168
İnsulin-2 mCi-37˚C	40,9 (±0,041)	41,0(0,34-0,47)	
Without insülin-10 mCi-37˚C	45,4 (±0,047)	46,0 (0,39-0,52)	0,575
İnsulin-10 mCi-37˚C	46,1 (±0,069)	45,0 (0,36 -0,58)	
Without insulin-2 mCi-25˚C Oda ısısı	33,0 (±0,067)	34,0 (0,23-0,41)	0,574
İnsulin-2 mCi-25˚C	33,6 (± 0,045)	34,0 (0,27-0,40)	

Table-3: Effect of different doses of F-18 FDG on leukocyte labeling efficiency of F-18 FDG

	Ortalama (±SD) (%)	Ortanca (min-maks.) (%)	P value
Without insülin-2 mCi	40 1 (±0,040)	40.0 (0,34–0,45)	**0,049**
Without insülin-10 mCi	45,4 (±0,047)	46.0 (0,39-0,52)	
Insulin-2 mCi	40,9 (±0,041)	41.0 (0,34 -0,47)	0,090
Insulin-10 mCi	46,1 (±0,069)	45.0 (0,36 -0,58)	

Table-4: Effect of incubation temperature on F-18 FDG leukocyte labeling efficiency

	Mean (±SD) (%)	Median (min-maks.) (%)	P value
Without insülin-25 C	33 (±0,067)	34 (0,23-0,41)	**0,042**
Without insülin-37°C	40 (±0,040)	40 (0,34–0,45)	
Insulin-25 C	33,6 (± 0,045)	34 (0,27-0,40)	**0,036**
Insulin-37°C	40,9 (±0,041)	41 (0,34 -0,47)	

In our study, we investigated the effect of dose difference by working with 2 mCi and 10 mCi F-18 FDG, and formed two separate groups with and without insulin addition in both groups. In the insulin-free group, we found that F-18 FDG leukocyte labeling efficiency was significantly higher in the high-dose (10 mCi) group than in the low-dose (2 mCi) group. No statistically significant difference was found between the F-18 FDG doses in the insulin-supplemented group. In the presence of insulin, the percentage of leukocytes binding with F-18 FDG was independent of F-18 FDG dose. Fostrom et al [15] labeled leukocytes with activity at different doses of F-18 FDG (100 microCuri, 500 microCuri, 1 mCi and 2 mCi) and reported no statistically significant difference between F-18 FDG doses in terms of labeled efficiency. Considering the long duration of labeling and the degradation of F-18 FDG, this may be caused by the fact that the activities used are very small and the difference between doses is very small.
In both the presence of insulin and the absence of insulin, the labeled efficiency obtained at 37˚C was significantly higher than the room temperature. In one study, F-18 FDG leukocyte labeling was performed at 20˚C and 37˚C. The labeling efficiency obtained at 37˚C was statistically significantly higher than 20˚C [15]. This result is consistent with our findings.

5. Conclusion

In our study, it was demonstrated that insulin has no effect on the labeling efficiency of F-18 FDG-leukocyte labeling in vitro. Labeling efficiency was found to be increased when labeling at body temperature (37˚C) and high F-18 FDG dose. The study appears to provide more accurate and clear information when repeated at 37˚C and with high F-18 FDG activity and blood volumes. Further studies with more patients are needed to explain this situation.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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