International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 2 (9), pp. 001-010, September, 2011. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Author(s) retain the copyright of this article.

*Full Length Research Paper*

# Clinical evaluation of $^{99m}$Tc -2IT-INH in normal subjects and patients with tubercular lesions

**Namrata Singh[1,2]* and Aseem Bhatnagar[1]**

[1]Institute of Nuclear Medicine and Allied Sciences, New Delhi - 110054, India.

[2]Ambedkar Center for Biomedical Research, University of Delhi, Delhi - 110007, India.

Accepted 11 March, 2011

**$^{99m}$Tc-INH of high labeling efficiency and stability has been developed using indirect method. *In vitro* studies and animal experiments indicated its advantages as a specific tuberculosis imaging agent. The objective of this study was to establish the efficacy of $^{99m}$Tc-INH in humans with sensitive as well as resistant tuberculosis by conducting a phase I clinical trial. The biodistribution studies were done in normal subjects and phase I clinical trial was conducted in 20 patients. Whole body scan and spots were acquired at 1 and 4 h. Angiography, blood pool and 24 h spot images of the lesion bearing areas were also acquired. The biodistribution suggested absence of *in vivo* breakdown of radiotracer, with main excretory pathways being hepatobiliary and renal. The biodistribution of $^{99m}$Tc-INH was similar to the unlabeled INH reported earlier. Out of 20 patients, 13 patients with sensitive tubercular lesions in the lungs or bone and 2 patients with resistant tubercular lesion in lungs concentrated the $^{99m}$Tc-INH while in the other 5 cases with old healed lesions no concentration of $^{99m}$Tc-INH was observed in scintigraphy. An unsuspected bony lesion was discovered in a patient with known pulmonary disease. Bone lesions were visualized within 1 h while pulmonary lesions accumulated $^{99m}$Tc-INH very slowly with time and 24 h acquisition appeared essential for the diagnostic interpretation. No adverse reaction was observed in the patients post injection. $^{99m}$Tc-INH developed is safe for human use and has potential to qualify as a specific tuberculosis imaging radiopharmaceutical.**

**Key words:** Isoniazid (INH), $^{99m}$Tc, scintigraphy, clinical trials, radiopharmaceutical.

## INTRODUCTION

Development and clinical evaluation of specific radiopharmaceuticals for infection imaging is an important area of research in nuclear medicine. The current focus is on infection imaging, particularly tubercular, since it is widely recognized as an area where our diagnostic capability is rather limited despite major advances. The objective of current reasearch in this area is to identify non-invasive and easy to perform techniques of high diagnostic accuracy. Localization of site infection is also a priority, in order to obtain samples to confirm diagnosis and resistance profile of the infecting bacteria, to assess the treatment response objectively and toconsider surgical intervention wherever appropriate.

Radiological imaging is preferred in clinical practice, predominantly because it is ubiquitous (X-ray, ultrasound and CT scan), and has fine resolution (CT and MRI). Scintigraphy is now recognized as a tool for *in vivo* diagnosis of bacterial infection due to its sensitivity, spe-cificity and whole body facility when the probable site of infection is unknown. SPECT agents like radiolabeled white blood cells (the 'old warhorse') (Michael, 1994; Datz, 1994; Cook et al., 1984), $^{99m}$Tc -Ciprofloxacin (Vinja-muri et al., 1996; Hall et al., 1998; Britton et al., 2002) and FDG, a PET radiopharmaceutical (Barkeet et al., 2000; Cheery 2001), are now considered 'routine' bacte-rium-specific-radiopharmaceuticals, espitepersisting de-bates, mainly focused on interpretation of the scan rather than the techniques perse. Earlier, non-specific inflamma-tion imaging radiopharmaceuticals like $^{99m}$Tc-Dextran pro-ved useful in locating intestinal tubercular ulcers by virtue of their bleeding and associated protein losing enteropathy (Bhatnagar et al., 1999). $^{99m}$Tc-Ciprofloxacin

*Corresponding author. E-mail: namsingh1@yahoo.com or singhnamrata2009@gmail.com. Tel: +91-9810388574.

was also used for tuberculosis detection. An Indian multi-centric trial was initiated by developing, evaluating, and distributing indigenous ciprofloxacin for research purpo-ses (Britton et al., 2002; Bhatnagar et al., 1999; Singh et al., 2002). The diagnostic capability of $^{99m}$Tc-Ciprofloxacin was clinically acceptable in peripheral tuberculosis, ost-eomyelitis and prosthesis infection, but its sensitivity in soft-tissue tuberculosis (pulmonary, cervical and abdomi-nal lymphadenopathy), bone tuberculosis of the central skeleton and cervical tubercular nodes was limited. Therefore, search for its true value as a specific bacterial radiotracer continues as some investigators have found it to possess very high sensitivity-specificity even for soft-tissue tuberculosis (Britton et al., 2002) while other have found it rather non-specific with unacceptably low spe-cificity (Beckerman, 1988; Buscombe et al, 1990). $^{99m}$Tc-MIBI (Onsel et al., 1998) and $^{99m}$Tc-Tetrafosmine (Derir-menci et al., 1998) showed concentration in tubercular lesion by means of non-specific uptake but these radio-tracers had limited clinical success. Based on these facts, $^{99m}$Tc-Isoniazid (INH), an antimicrobial specific to tuber-culosis was developed. An indirect method was used to radiolabel the antimicrobial with Tc-99m (Derirmenci et al., 1998). An excellent radiolabeling (> 95%) (Singh et al., 2003) and high *in vitro* and *in vivo* stability of the $^{99m}$Tc-INH was obtained. After obtaining a proof of its specific interaction with human isolates of mycobacterium tuberculosis and its intact biological activity following radiolabeling, relevant animal data was collected. These data suggested that the diagnostic test may be safe for use and the organ distribution and other parameters were consistent and bore a correlation with the original drug (Singh et al., 2003; Dollery, 1998) . This communication reports the further progress done on the subject. The objectives of this paper are to conduct initial human trials and to compare data obtained with $^{99m}$Tc-INH in living systems with the known behavior of the original drug in the human system.

## MATERIALS AND METHODS

### Radiocomplexation of INH

INH salt was procured from Sigma chemicals. Radiocomplexation of INH was done by the method reported earlier in our lab (Mathew et al., 2000; Singh et al., 2003). Briefly, INH was derivatized using 2-iminothiolane to introduce exogenous sulfhydryl group. The reaction took place in one and a half hour of incubation of the above solution in triethanolamine with subsequent addition of chloroform. Stannous tartrate was added to the derivatized INH and aliquots were prepared after passing the contents through a sterile Millipore filter (0.22 µ). Each unit had enough derivatized reduced INH to react with up to 400 MBq of Tc-99m pertechnetate at the time of use. Physiochemical characterization of $^{99m}$Tc-INH was done by infrared and mass spectrum studies. Good manufacturing practice measures were followed strictly, using fresh chemicals and sterile laboratory ware. If the labeling efficiency was > 95%, $^{99m}$Tc-INH was used for further studies.

### *In vitro* and *in vivo* studies

*In vitro* serum and blood stability studies of $^{99m}$Tc-INH were carried out by mixing $^{99m}$Tc- INH with serum and blood seperately. 2 l of samples were taken out from each vial at 1, 4 and 24 h of incu-bation and were assessed by ITLC system seperately. Experi-ments were carried out in compliance with the relevant national laws relating to the conduct of animal experimentation. $^{99m}$Tc-INH was used further in pharmacokinetic and biodistribution studies in New-Zealand white rabbits and Balb/c mice respectively. Para-meters like plasma clearance; half life, plasma protein binding and volume of distribution were calculated and compared with unlabeled INH. All tests were done in triplicate. The results were evaluated quantitatively and qualitatively. *In vitro* pharmacological studies that are MIC (Minimum inhibitory concentration) experiment, drug uptake studies, colony forming assay (CFU assay) were done.

### Human studies

### Study design

The stated objectives of the phase I clinical trial were; to confirm safety aspects of the new radiopharmaceutical in humans, to check consistency and uniformity of behavior of $^{99m}$Tc-INH in humans, to suggest a practical study protocol and to study its uptake in known active and inactive tubercular lesions. The patients were either freshly diagnosed (on no treatment, or on ATT for a very short period) or had ‘cured’ or inactive tubercular lesions. As per plan 2 normal subjectss and 20 patients were chosen for the study.

### Patient selection

Institutional ethical committee permission was taken prior to initiation of the human studies. The Two subjects were healthy nor-mal subjects without any known disease for biodistribution studies. The next 20 subjects were ambulatory patients with proven tuber-culosis with known sites of infection. Out of which 8 patients had bone tuberculosis; 5 had pulmonary fresh lesions (proof of infection and site identification by radiology and serology in clinical context), 2 had pulmonary resistant tuberculosis and 5 had old dormant lesions. Only those patients were recruited for the study who were not overtly sick and did not suffer from any other disease or disorder. One of the patients was a child and was only recruited on specific request of the physician in-charge, as it was felt that scintigraphy might provide requisite diagnostic help. As a part of abundant precaution, the patients were admitted for at least a day for observation though no detailed investigations were done for creating detailed toxicity data profile of $^{99m}$Tc-INH. Details of the patients and the outcome are presented in Table 1.

### Scintigraphy procedure

There was no food restriction on the patients. 370 MBq of freshly prepared $^{99m}$Tc-INH was injected intravenously as a slow bolus in adults, with correspondingly reduced dose in the child. Angiography and blood pool phase were acquired as per requirement, focused over organ/site known to harbor tubercular lesion. Spot acquisitions of all sites known to harbor tubercular lesions were taken in the

**Table 1**. Biodistribution data of $^{99m}$Tc-INH in Balb/c mice.The mice were administered 40KBq of $^{99m}$Tc-INH and theradioactivity in different organs were measured at 1, 4 and 24 h respectively. Each value is the mean ± SD of three mice and expressed as percentage administered dose per gram organ

| Organ/Tissue | 1 hour | 4 hours | 24 hours |
|---|---|---|---|
| Blood | 1.5± 0.02 | 1.3± 0.24 | 0.8± 0.02 |
| Heart | 0.95± 0.21 | 1.1± 0.07 | 0.74±0.6 |
| Lungs | 2.1± 0.18 | 1.88± 0.2 | 1.05± 0.04 |
| Liver | 7.2± 0.3 | 8.0± 0 | 3.8± 0.41 |
| Spleen | 2.8± 0.19 | 3.3± 0.43 | 2.56± 0.02 |
| Intestine | 6.4± 0.8 | 5.62± 0.07 | 1.03± 0.03 |
| Kidneys | 12.6± 0.42 | 10.3± 0.32 | 2.02± 0.04 |
| Muscle | 0.39± 0.2 | 0.48± 0.2 | 0.13± 1.1 |
| Bone | 1.04± 0.28 | 2.0± 1 | 1.0± 0.05 |
| Brain | 1.01± 0.06 | 0.78± 0.19 | 0.69± 0.2 |
| Stomach | 0.85± 0.31 | 0.75± 1 | 0.85± 0.3 |

**Table 2**. Comparison of derived parameters i.e. $T_{1/2}$, half life; $V_d$, volume of distribution; Cl, clearance of $^{99m}$Tc-2IT-INH and INH. Pharmacokinetic study of $^{99m}$Tc- INH was done in New Zealand white rabbits. Rabbits were administered i.v. with 37MBq $^{99m}$Tc -INH. The blood was withdrawn at different time intervals and radioactivity was measured. Data was expressed as percent of radioactivity in whole body blood, taking 7% of body weight as blood.

| Parameters | $^{99m}$Tc-2IT-INH | INH |
|---|---|---|
| $T_{1/2}$ (hrs) | 4.8 | 2-6.5 |
| $V_d$ (L/Kg) | 0.76 | 0.6-0.81 |
| Cl (ml/hr) | 46 | 35-50 |

best view using a standard dual-head gamma camera. Counts acquired were kept flexible and depended on the site of lesions. Imaging of all known lesions was done at 1, 4 and 24 h of post injection. Anterior and posterior spots of abdomen and chest were also acquired at 24 h to aid biodistribution assessment. Additional images were acquired as per camera time availability. Whole Body acquisitions were acquired at 1 and 4 h at slow speed (10-15 cm/min). Additionally, SPECT data was acquired in three cases, one case with bone lesions at 6 h and the other two with pulmonary lesions. The patients were critically observed for any signs and symptoms and any deviation in the vitals throughout the day.

## RESULT

The labeling efficiency of $^{99m}$Tc-INH was found to be >95%. Only 2-3%of technetium leached out from $^{99m}$Tc-INH until 24 h of incubation of $^{99m}$Tc-INH in serum as well as blood *in vitro*. The tentative structure and charge of $^{99m}$Tc-INH was determined by physiochemical study.The IR spectra of unlabeled INH gave a peak at ~ 1668 cm$^{-1}$ due to C=O bond. NH and CH gave band at the region ~ 3014 cm$^{-1}$. IR of 2-IT-INH showed band at ~ 2360 cm$^{-1}$ due to exogenous -SH group. The peak at ~ 2360 cm$^{-1}$ disappeared in $^{99m}$Tc-INH indicated that Tc-99 m binds to sulphur via deprotonation of -SH group. Mass spectrum of unlabeled INH showed peak at 137.2, indicating its molecular weight. An additional peak at 273.1 was observed in 2-IT-INH due to binding of unlabeled INH with 2-iminothiolane. Biodistribution study data indicated maximum accumulation of $^{99m}$Tc-INH in kidneys followed by liver and intestine (Singh et al., 2003) indicating renal and hepatobiliary route of excretion (Table 1). Pharmacokinetic tudy data of $^{99m}$Tc- INH was comparable to unla beled INH (Table 2). *In vitro* pharmacological studies in- dicated retention of biological activity of INH after radio complexation. 90% of $^{99m}$Tc-INH was bound to plasma protein which reduced to 85% at 24 h.Scintigraphy done in rabbits at 1 and 4 h post injection showed high $^{99m}$Tc-INH uptake in the kidneys and liver (Mathew et al., 2000). Intestinal activity was evident signifying gall bladder exc retion. No activity was seen in the stomach region (Singh et al., 2003) indicating i*n vivo* stability of $^{99m}$Tc-INH.

### Scintigraphy and patient studies

### Safety aspects

No adverse effects or allergic reactions were noted in the

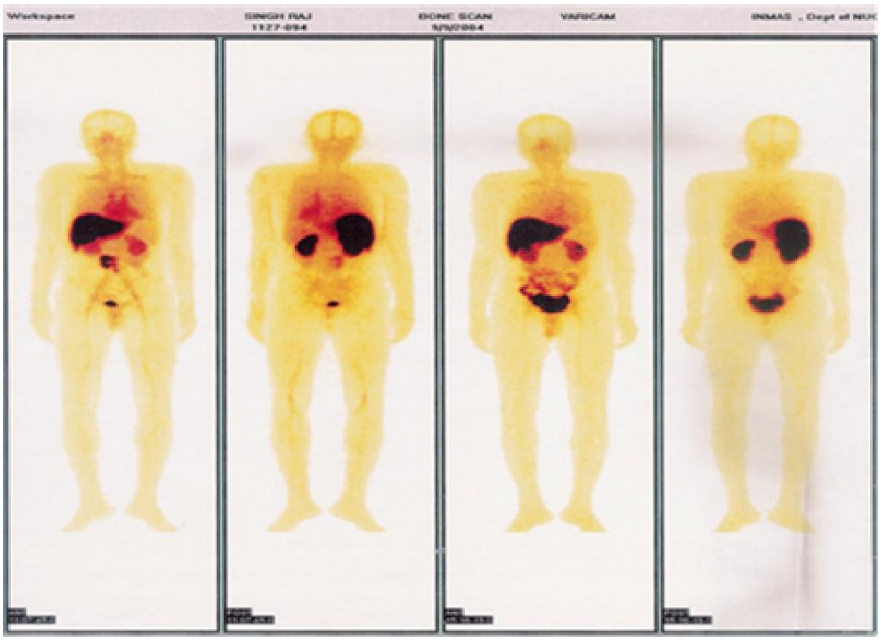


**Figure 1**. Biodistribution of $^{99m}$Tc-INH in human normal subjects 1 h anterior (A1) and posterior (A2) and 4 h anterior (B1) and posterior (B2).

patients in the immediate and acute period following tracer injection. The vital signs were checked periodically which remained stable. The patients were kept under observation overnight and did not report any problem. Since the drug molecule and the reducing agent (stannous tartrate) are well known clinical entities, used in complex formation is minute diagnostic doses, detailed toxicity data were not created.

## Human biodistribution of $^{99m}$Tc-INH

Whole body scans taken at 1, 4 and 24 h and at other convenient times were analyzed qualitatively. The tracer was well distributed in the body compartments by 1 h (except the blood brain barrier which was never brea-ched) (Figure 1), with marginally higher concentration in blood as shown by faint cardiac and great veins visua-lization. Hepatic accumulation of radioactivity appeared to increase slowly with time and later reduce, probably after 4 h. Intestinal visualization of radioactivity was mostly evi-dent before 4 h with frequent gall-bladder visualization. Significant urinary activity was seen by 1 h and kidneys were the main excretory organ. Significant renal cortex retention was also a constant finding. Appreciable lung uptake was seen in all subjects irrespective of presence of pulmonary disease. The uptake was fairly uniform in both lungs and appeared to buildup with time, probably stabilizing by 4 h, and reducing thereafter in 24 h. There was no bone or bone marrow activity, even in young subjects. Interestingly, no epiphysis activity was seen in the child (Figure 2). Thyroid and stomach were seen faintly in one case. Biodistribution in the child was not different to the adult counterparts.

## Study protocol and Lesion uptake

A total of 20 lesions were identified as infected as a pre-scan diagnosis. Out of which 7 had pulmonary (soft-tissue) and 8 had bone lesions in peripheral skeleton and rest 5 had treated inactive pulmonary lesions (Table 3). Soft-tissue lesions known to be infected with sensitive tuberculosis gained in radioactivity with time (Figure 3). A majority of soft-tissue lesions were actually not visualized at all before 4 h of post injection; one was visualized only at 24 h. interestingly, an unsuspected pulmonary lession was discovered on one scan that was later confirmed a fresh x-ray chest. SPECT acquisitions done on pulmonary lesions seen in planar view in two patients were of poor quality and failed to locate the lesion with any defini-tion or contrast. All lung lesions, where angiography and blood-pool imaging was performed, remained non-visualized on these acquisitions.Resistant tubercular lesion was observed at 1 h of post injection of $^{99m}$Tc-INH and the significant radioactivity was observed until 24 h (Figu-

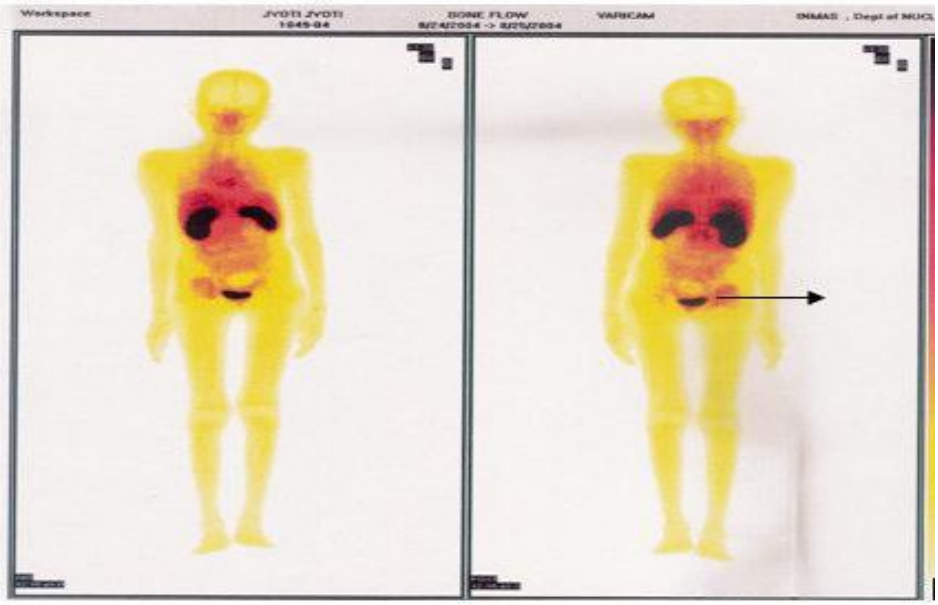

**Figure 2.** Whole body scan of anterior (A) and posterior view (B) in 12 years old child with tubercular lesion ( → ) in pelvic bone at 1 h post injection of 370MBq of $^{99m}$Tc-INH.

re 4). Bone tubercular lesions were well visualized even on one- hour scan, and appeared to gain little activity thereafter; however no radioactivity appeared to be lost either (Figure 5). Thus, there was a very slow ratio enhancement with time. In general, abnormal tracer uptake in bone lesions was of a quantitatively higher order compared to the lung lesions, which required higher count collection and delayed acquisition for proper visualization.

## DISCUSSION

### Safety and toxicity

Each vial contains 2 mg INH and up to 40 µg of stannous tartrate with known safety profile. Adverse effects of INH, like hepatotoxity and neurotoxicity are dose dependent, occurring in a few cases after several months of therapeutic dose. So, the diagnostic dose used was inconsequential. Stannous tartrate is used in many radiopharmaceutical kits in similar doses. Iminothiolane was used as an intermediate chemical for creating sulphhydryl group, is an inseparable part of the technique used before also which gets washed out of the injectable system by repeated washings at the time of its preparation. In clinical context, our experience suggests that it should be a safe radiopharmaceutical.

### Biodistribution of $^{99m}$Tc-INH in humans

Biodistribution of $^{99m}$Tc-INH in humans was comparable to the mice (Singh et al., 2003) and unlabeled INH showing maximum uptake of $^{99m}$Tc-INH in kidneys followed by liver and intestine. Neutral charge on $^{99m}$Tc -INH and single moiety presentation on IR spectrum as well as intact biological activity (Degirmenci et al., 1998; Singh et al., 2003) explains this equivalence. In a small series, however, this equivalence may not be there (even if behavior of the ‘hot’ and ‘cold’ entities is same) due to almost equal frequency of fast and slow ‘acetylators’in the population. Blood clearance of INH may be 5 times faster in ‘fast’ acetylators (Black, 1974), causing this dis-crepancy. It is therefore expected that soft- tissue back-ground radioactivity may clear significantly faster in few patients after $^{99m}$Tc-INH injection and modification in acquisition protocol may have to be made to maintain high diagnostic value.INH is mainly metabolized in liver and intestinal wall. There are several metabolites that are excreted mainly through renal pathway, a minority being routed through biliary system to faeces (Black, 1974; Elmendorf et al., 2001). This explains the slow buildup of radioactivity in liver parenchyma with subsequent plateauing or outflow from liver and correlates well with absence of radiocolloids in the injected radiopharmaceutical. Signifi-

**Table 3.** Clinical details of patients and result of $^{99m}$Tc-INH scintigraphy.

| Sex/ age | Clinical diagnosis | Site of lesion | Treatment history | Outcome |
|---|---|---|---|---|
| Male/30 | Bone TB | Ankle | Tuberculosis of ankle, active on MRI clinically. | True positive. |
| Female/12 | Bone TB | Spine and acetabulaum | Active pott's spine.<br>Psoas abscess (2).<br>Whole body scan showed unsuspected pelvic region confirmed later in MRI (4 lesions) | True positive. |
| Male/41 | Bone TB | Spine | Active vertebral abscesses | True positive. |
| Male/34 | Bone TB | Spine | Carries sine $C_4$-$C_5$ retropharyngeal abscesses.<br>MRI carries yellow marrow replacement in vertebral bodies. | True Positive. |
| Male/28 | Bone TB | Spine | Tuberculosis of spine, active on MRI clinically. | True positive. |
| Female/40 | Bone TB | Ankle | Tuberculosis of ankle, active on MRI clinically.<br>Positive in 4$^{th}$ metatarsal right foot | True positive. |
| Female/33 | Bone TB | Spine | Abcesses in $C_3$-$C_4$ cervical spine | True positive. |
| Female/36 | Bone TB | Lymph nodesin neck | Calcified neck region | True positive. |
| Male/50 | Sensitive pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Male/34 | Sensitive pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Male/50 | Sensitive pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Male/29 | Sensitive pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Female/23 | Sensitive pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Female/30 | Resistant pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Female/42 | Resistant pulmonary TB | Lungs | Active pulmonary tuberculosis | True positive. |
| Male/35 | Pulmonary TB | Lungs | Old treated case with 3 years on ATT treatment | True negative |
| Male/40 | Bone TB | Spine | Old treated case of Pott's spine ($T_7$-$T_9$) with local tenderness | True negative |
| Male/53 | Bone TB | Spine | Old treated case of cervical TB with local tenderness | True negative |
| Female/37 | Pulmonary TB | Lungs | Old treated case with 1.5 years on ATT treatment | True negative |
| Female/30 | Bone TB and pulmonary TB | Lymph nodesIn neck and chest region | Calcification in left hilar region with no ATT treatment | True negative |

cant intestinal activity noted both in animals and humans may be due to excretion by gall bladder and also in part due to intestine being a site participating in metabolism of INH. Early visualization of radioactivity in urine is explained by the fact that a small part of INH excreted in urine was unmetabolized (Elmendorf et al., 2001). We, however, do not know the chemical nature of entities binding radioactivity in blood and urine after catabolism of INH, which is quite fast. The fact that free pertechnetate is not released in blood after metabolism, at least not until 24 h, helps make this radiotracer potentially useful for imaging tubercular lesions. INH is not known to selectively collect in or interacts with reticulo-endothelial system and bone.

Correspondingly, $^{99m}$Tc-INH was not found to accumulate in spleen, bone marrow and bone, which is a distinct

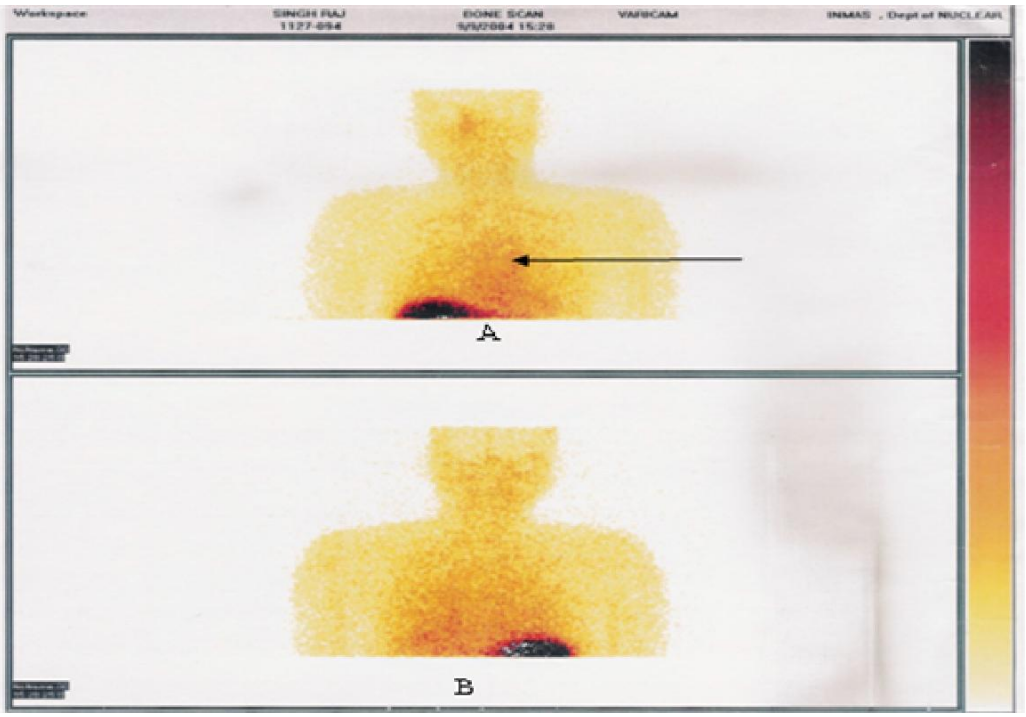


**Figure 3**. Scintigrams of a patient showing preferential localization of $^{99m}$Tc-INH in the sensitive pulmonary lesion at 1 h(A) and 4 h (B) post injection of 370MBq of $^{99m}$Tc-INH.

advantage and may add specificity to diagnosis of bony lesions. Non-accumulation of the tracer in epiphysis pla-tes in the child is an important negative observation, because it suggests high specificity of $^{99m}$Tc- INH in com-parison to agents like $^{99m}$Tc-Ciprofloxacin, which are known to show non-specific bone uptake. Interestingly, INH is known to accumulate in lung parenchyma, which probably serves as its depot (Elmendorf et al., 2001; Alfonso, 2001; Barclay et al., 1953). This was observed consistently with $^{99m}$Tc-INH (without previously knowing that it was the expected pharmacological behaviour) which was initially unexplainable in absence of macro-colloids in the injected preparation. We believe it gives distinct advantage to $^{99m}$Tc-INH as a specific tuberculosis imaging agent because poor bacterial count, low meta-bolic status of the bacteria, and fibrotic encapsulation of tubercular lesions in the lungs suggests relatively poor sensitivity of any Tc- 99 m based radiotracer. $^{99m}$Tc-INH is in an advantageous position compared to other specific tracers because a higher physiologic concentration of the radiolabeled drug in the lung parenchyma may translate into higher uptake by mycobacterium, and therefore into higher sensitivity of detection of pulmonary lesion. For this reason, delayed imaging until 24 h appears essential for imaging. There appear to be a few areas of discordance between the *in vivo* behavior of $^{99m}$Tc -INH and unlabeled INH. The protein binding of the native drug is negligible while that of the radiolabeled form was around 90%, this is at logical variance with our data that show fair correspondence of plasma clearance rate and volume of distribution between the two forms of the drug. We believe this discrepancy can be explained if it is assumed that binding of the radiolabeled drug with plasma proteins is trivial and readily breaks down with release of the parent molecule *in vivo*. However, more evidence needs to be collected in this area. Secondly, less than 10% of the unlabeled drug is excreted through hepatobiliary route; qualitative analysis of scans suggests that a more significant amount of radioactivity passes through the biliary route. It is possible that the radiolabeled metabolites produced in liver are non-polar and prefer biliary route rather than the urinary route. Our observation that gall bladder and/or intestines are visualized quite late in the study support this hypothesis.

## Tubercular lesions on $^{99m}$Tc-INH imaging

The purpose of this preliminary human study was to evaluate feasibility of using the radiotracer in humans for tuberculosis scintigraphy. Therefore, the uptake patterns observed in the known lesions are mainly focused in this work rather than hypothesizing the diagnostic accuracy of the radiotracer. $^{99m}$Tc-INH was first prepared in 1987 by a Japanese group (Yamada et al., 1987) and used for tu-

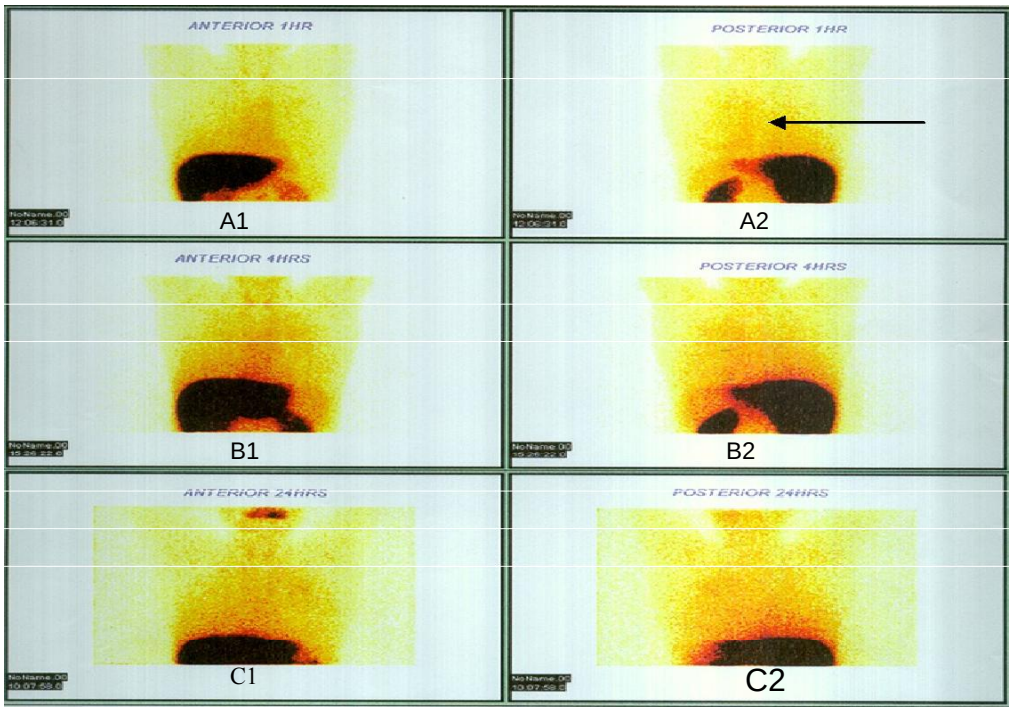


**Figure 4**. Scintigrams of human showing preferential localization of $^{99m}$Tc-INH in the resistant pulmonary lesion ( → ) at 1 h anterior (A1) and posterior view (A2), 4 h anterior (B1) and posterior view (B2) and 24 h anterior (C1) and posterior view (C2) post injection of 370MBq of $^{99m}$Tc-INH.

mor imaging. The approach was discarded because radiolabeling achieved was not optimum. Our attempt therefore was to use the tracer in humans for tubercular imaging. Our results suggest that $^{99m}$Tc-INH shows speci-fic uptake in tubercular lesions, both in the soft-tissue les-ion as well as the peripheral skeleton. In soft tissue, the uptake is very low or absent in the initial one hour and tends to increase with time. Any soft- tissue lesion in the angiography or blood pool phase was not visualized pro-bably because of lack of typical inflammatory response as seen in acuteinfections. Perfusion and blood-pool pha-se thereforeoccurs at 4 h and starts reducing thereafter. This suggests thatincrease in target-to-non target ratio beyond 4 h in predominantly due to reducing background. The relatively short biological half-life of $^{99m}$Tc-INH is therefore a contributory factor in enhancing sensitivity of the tracer, partially offsetting slow and reduced bacterial uptake of the tracer. The tracer uptake in pulmonary les-ions was visualized only at four hours many times and sometimes at 24 h, which suggests that 24 h imaging is essential in imaging soft tissue lesions. Uptake ratio in the lesions with respect to background appeared to be around 1.2-1.3 by four hours, that is, barely enough for visual appreciation. We now recommend high count ima-ging of the soft-tissue lesions at 6 and 24 h, other acqui-sition times being optional. In our experience SPECT imaging has not be useful in pulmonary tuberculosis because of low tracer concentration in lesions in the initial four hours, while both physical decay and low uptake contributes to poor contrast and indefinite lesion contours. Multi-dimensional views obtained late in the day will probably provide best view to the lesion. In peripheral bone tuberculosis, the problem of reducedand delayed uptake does not appear to be significant.Angiography and blood pool phases were positive in all cases; nil or minimal rise in uptake ratio occurred between 1 and 4 h. This suggests that 24 h imaging may not be necessary in bone tuberculosis. The reason of higher uptake of the tracer in bone lesions (compared to pulmonary lesions) appears to be microfractures caused in and around bone lesions by pressure effect of the lesion. Repair of these microfractures causes increased vascularity thus bringing in more radiotracer per unit volume in the lesion (similar to higher uptake of Tc-99m MDP in bone metastases).

In conclusion, the present study suggests high labeling efficiency, *in vitro* and *in vivo* stability and uniform human biodistribution of $^{99m}$Tc-INH similar to the parent molecule. It appears safe for human use, with low possibility of allergic or antigenic response, or interference with therapeutic doses. The $^{99m}$Tc-INH may be sensitive in both pulmonary and peripheral forms of the infection including resistant tuberculosis. More studies are needed to evalu-

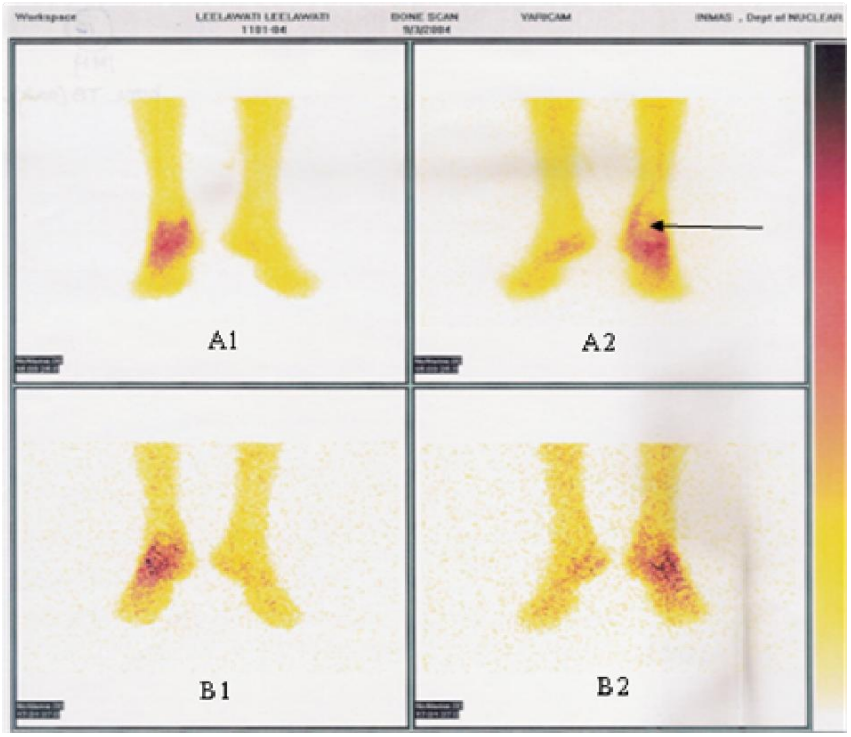


**Figure 5.** Scintigrams of human showing preferential localization of $^{99m}$Tc-INH in the bone lesion in ankle ( → ) at 1 h anterior (A1) and posterior view (A2), 4 h anterior (B1) and posterior view (B2) post injection of 370MBq of $^{99m}$Tc-INH.

ate the diagnostic efficacy of $^{99m}$Tc-INH in MDR tuberculosis. Negligible uptake in osteoblastic bone (uninfected) suggests high specificity of $^{99m}$Tc-INH in bone lesions, an area where $^{99m}$Tc- Ciprofloxacin appears to be lacking. We are now planning further study of $^{99m}$Tc-INH, in compari-son with the conventional tracers like $^{99m}$Tc-MDP and $^{99m}$Tc- ciprofloxacin, to extend its use to central skeleton and non-pulmonary soft-tissue lesion.

## ACKNOWLEDGMENTS

I am thankful to Maj. Gen., T. Ravindranath and Dr. A.K Singh for providing me guidance to complete this work.

### REFERENCES

Alfonso RG (2001). Remington's pharmaceutical sciences. Harvey SC, Withrow CD. Basic pharmacokinetics. Mack Publishing Company Easton, Pennsylvannia. 18th Edition. 725-745.

Bakheet SM, Powe J, Kandil A, Ezzat A, Rostom A, Amartey J (1953) F-18 FDG uptake in breast infection and inflammation. *Clin Nucl Med* 2000; 25(2): 100-103.

Barclay WR, Ebert RH, LeRoy GV, Manthei RW. Distribution and excretion of radioactive Isoniazid in Tuberculosis patients. JAMA. 151(6): 1384 -1388.

Beckerman C (1988). Gallium-67 Scanning in clinical evaluation of human immunodeficiency virus infection: indications and limitations. Semin Nucl Med. 18 :273.

Bhatnagar A, Jain CM, Kashyap R, Singh AK, Gupta A, Chopra MK, Swaroop K (1999). A comparative study of $^{99m}$Tc-infecton, $^{99m}$Tc-Human ImmunoglobulinG and $^{99m}$Tc-Dextran in tubercular bone disease. IJNM.14(1): 10-16.

Bhatnagar A, Sharma, R, Gupta S, Singh AK, Soni NL, Rawat H (1999). Possible role of $^{99m}$Tc-Dextran($^{99m}$ Tc-DX) in detection of occult small intestinal lesion. IJNM. 14: 30 - 32.

Black M, Isoniazid and Liver (1974). Am. Rev. Respir. Dis. 110: 1-3.

Britton, KE, Wareham, DW, Dass, S, Solanki, KK (2002). Imaging bacterial infection with 99mTc-ciprofloxacin. J. Clin. Pathol. 55(11): 817-823.

Buscombe JR, Lui D, Ensing G, Jong De. and EII PJ (1990). $^{99m}$Tc-human immunoglobulin first result of new agent for localization of infection and inflammation. Eur. J. Nucl. Med. 16: 649-655.

Cheery SR (2001). Fundamentals of positron emission tomography and application in preclinical drug development. J. Clin. Pharmacol. 41(5): 482-491.

Cook PS, Datz FL, Disbro, MA *et al* (1984). Pulmonary uptake of $^{111}$Leucocyte imaging. Clinical significance in patient with suspected occult infections. Radiology. 150 : 557-561.

Datz L. Fredrick (1994). Indium-111-Labelled Leucocytes for the detection of Infection: Current status. Sem. Nucl, Med. 24(2): 92-109.

Degirmenci, Kiline O, Cirak KA, Akpinar O, Halilcolar H, Durak H (1998). $^{99m}$ Tc-tetrofosmin scintigraphy in pulmonary tuberculosis. J. Nucl. Med. 39: 2116-2120.

Dollery C (1998). Therapeutic Drugs. Churchill Livingstone. 2: 1-75.

Elmendorf DF, Jr, Cawthon WU, Muschenheim C, Dermott WM (2001).The absorption, distribution, excretion and short-term toxicity of isonicotinic acid hydrazid (Nydrazid) in man. Amer. Rev. Tubercl. 65: 429-442.

Hall, AV, Solanki, KK, Vinjamuri, S *et al* (1998). Evaluation of the efficacy of "$^{99m}$Tc-infecton": A novel agent for detecting sites of

infection. J. Clin. Pathol. 51: 215-219.

Michael P (1994).The utility of [$^{99m}$Tc]-HMPAO-Leucocytes for imaging infection. Sem Nucl Med. 24(2): 110-127.

Onsel C, Sonmezoglu K, Camsari G et al (1998). Techetium-99m MIBI scintigraphy in pulmonary tuberculosis. IJNM. 39: 2116-2120.

Patent pending: Mathew TL, Singh AK, Verma J ((2000). A process patent entitled 'A process of preparation of Technetium 2-Iminothiolane Isonicotinic acid hydrazid complex and products thereof' must have been filled/granted in Indian Patent Office, New Delhi in May.

Singh AK, Bharadwaj N, Singh T, Kashyap R *et al* (2002). "Diagnobact" Single vial cold kit of $^{99m}$Tc-ciprofloxacin ready for multicentric trails. Abst. IJNM .17(4): RPS-16.

Singh AK, Verma J, Bhatnagar A, Sen S, Bose M (2003 ).Tc-99m Isoniazid: A specific agent for Diagnosis *of Tuberculosis.* WJNM. 4: 292-305.

Vinjamuri, S, Solanki, KK (1996). Comparison of Tc -99m infecton imaging with radiolabeled white cell imaging in the evaluation of bacterial infection. Lancet. 346 : 233-235.

Yamada N *et al* (1987). Investigation of $^{99m}$Tc-labelled tumour seeking agents.Usefulness of $^{99m}$Tc-INH radioisotopes. 36(10): 506-510