REVIEW ARTICLE

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Pharmacoscintigraphy: An Unexplored Modality in India

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Establishment of a new molecule as a therapeutic agent requires extensive inputs in terms of money, energy and time. Therefore the worldwide pharmaceutical R and D are focusing its attention on the development of new drug delivery systems. After designing a new delivery system, its optimization involves the *in vitro* and *in vivo* studies. Pharmacopoeial dissolution testing is an important *in vitro* test to study the release of the drug from its formulation. *In vivo* studies can be carried out either by blood sampling method or urine analysis. Besides, being cumbersome and tedious these methods do not give any information regarding the biopharmaceutics. Pharmacoscintigraphy, a much-touted technology can answer all above-mentioned queries regarding a delivery system. g-Emitting radionuclide (preferably ^{99m}Tc) is tagged with the active constituent or the excipient of the formulation. The radioactive dosage form is administered via intended route of administration and the subject (humans/animals) is scanned under a gamma camera. Radionuclides tagged with drugs/formulations/devices can provide vital information regarding the extent, rate, site, and mode of drug release and morphology of the drug delivery system during release in humans under the ethical norms. Pharmacodynamics and pharmacokinetics of new drug molecules studied by this technique gives qualitative as well as quantitative data. Present study proposes an effective approach for the development and evaluation of new drug molecules and drug formulations.

A new molecular entity (NME) either of natural or synthetic origin has to pass through a number of stages before being formulated into a delivery system. The preclinical evaluation of NME involves a study regarding its pharmacodynamics, pharmacokinetics (metabolism and excretion), toxicity, carcinogenecity, indications, contraindications, and interactions with drugs and food. But inadequate development chain, less preformulation research and lack of focus in clinical pharmacology leads to either premature closure or marketing failures. Establishment of a NME as a therapeutic agent requires extensive inputs in terms of money, energy and time. The much-touted figure of R and D expenditure for each NME is stated to be in excess of $ 500 million. The public citizen report from US has found out that actual after-tax cash outlay really spent on each molecule (including failures) is $110 million[1].

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Therefore, worldwide research is focusing its attention to the development of new drug delivery systems. Change in the mode of drug delivery can change the entire therapeutic potential of the drug. Scientists are trying to explore different routes for administration of a drug via new drug delivery systems. Sustained release drug delivery can decrease the dose and dosage frequency and hence increases the therapeutic efficacy. Development of colon specific delivery devices is useful for the delivery of peptides and proteins, which degrade, in acidic environment of stomach. Transdermal patches for drugs undergoing extensive first pass metabolism increase the bioavailibility of drugs. Aerosols via nasal mucosa can achieve the desired drug concentration in blood. Metered dose inhalers,

nebulisers and dry powder inhalers have carved a special place in the control of asthma.

PHARMACOSCINTIGRAPHY

Gamma scintigraphy is a well-established technique for the diagnosis of various diseases in neurology, oncology and cardiology. Pharmacoscintigraphy is the application of gamma scintigraphy technique. This technique is based on tracing the path of the radiolabelled ingredient of the formulation from the delivery system to the gastrointestinal tract, then into the systemic circulation. The radiolabelling of active ingredient or any excipient of the formulation can visualize the delivery system under the gamma camera (fig. 1). Pharmacoscintigraphy can be used in the evaluation of: a) new drug molecules, b) new drug delivery systems/ formulations and c) Therapeutic drug monitoring.

NMEs need to be evaluated for their distribution in the organs and their pharmacological responses at preclinical stage. Quantitative assessment of a molecule in organs is a cumbersome job. Moreover, biodistribution studies are feasible in only small animals at this stage. Pharmacoscintigraphy however takes an upperhand by making use of the radionuclide as a marker for the drug molecule in each organ and assessing the concentration of the drug by counting the radioactivity in the organs. The dynamic picture of the drug deposition and clearance from the organs can be studied by injecting the radiolabelled molecule intravenously into the subject and then imaging under gamma camera[2]. To study the pharmacological response the organ or the organ system can be highlighted

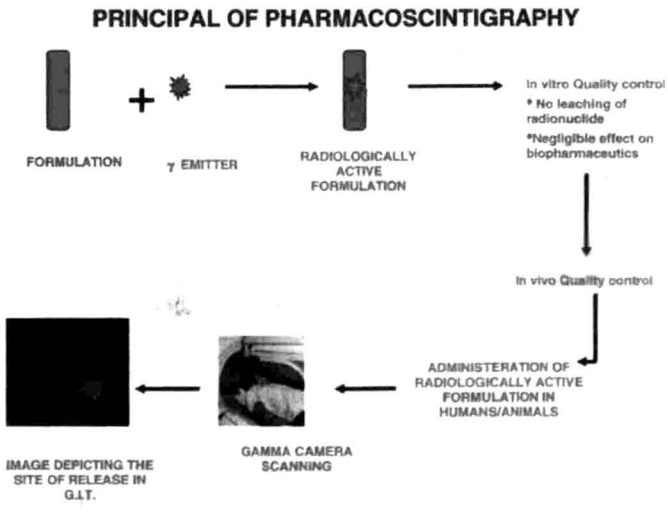

Fig. 1: Principle of pharmacoscintigraphy.

using a particular radiopharmaceutical. The change in the physiology and/or the morphology of the region of interest after the administration of the drug tells the pharmacological effects of the molecule.

Optimization of a new delivery system involves both *in vitro* and *in vivo* studies. Pharmacopoeial dissolution testing is an important *in vitro* test to study the release of the drug from its formulation. Although much about the performance of a system can be learned from *in vitro* release studies using conventional and modified dissolution methods, *in vivo* evaluation is essential in product development. *In vivo* studies can be carried out either by blood sampling method or urine analysis. Besides, being cumbersome and tedious these methods do not give any information regarding, a) The release of drug from formulation, b) Site of drug release, c) Morphology and fate of delivery system and d) Transit time.

The non-invasive technique of pharmacoscintigraphy can been used to follow the transit and release characteristics of a variety of pharmaceutical dosage forms[3]. Such studies provide an insight into the fate of the delivery system and its integrity and enable the relationship between *in vivo* performance and resultant pharmacokinetics to be examined.

Evaluation of the drug delivery systems/formulations can be carried out either by tagging the active ingredient or the excipient of the formulation or by studying the pharmacological response at the interested region.

Depending upon the information required for a molecule the radionuclide of a particular half-life is selected. The use of radionuclides such as ^{99m}Tc, 111-In, 131-I, 123-I, etc. in association with Single Photon Emission Computed Tomography (SPECT) can visualizes the site, pattern and morphology of delivery system. Pharmacoscintigraphy probably represents the only technique currently available for the quantification of drug release and pharmacokinetics in human or more specifically in the patient group[4]. Pharmacoscintigraphy has been used to investigate the pharmacology of the drug molecule and biopharmaceutics of a drug delivery system[5,6].

Apart from the pharmacoscintigraphy, positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) are gaining importance. PET makes use of the radionuclides of the physiological elements like carbon, oxygen, and nitrogen, which make this technique much more applicable in pharmacoscintigraphy[33].

PHARMACOSCINTIGRAPHY IN NEW DRUG DEVELOPMENT

The therapeutic potentials of NMEs can be explored at the pre-formulation stage, using gamma scintigraphy. Whole body imaging done periodically gives visual and quantitative information about its distribution, organ-specific accumulation (qualitative and quantitative) metabolic and excretory pattern.

After radiolabeling ciprofloxacin[7], sparfoxacin[8] and isoniazid[9] with ^{99m}Tc, we were able to establish their human (or animal) biodistribution pattern with ease, cost-effectivity, reproducibility and correlation with the corresponding lab data. Ciprofloxacin was found to get accumulated in epiphytic areas and kidneys as suggested by scintigrams.

We studied the pharmacological action of a plant extract using this technique. The aqueous extract of the *Podophyllum hexandrum* was evaluated for antiinflammatory action. ^{99m}Tc-dextran was used to highlight the sterile localized inflammation on the left thigh of the rabbit. ^{99m}Tc-dextran was administered intravenously to the rabbit and the scintigrams (fig. 2) of the inflammatory lesion were taken[10]. The potential of the plant extract as an antiinflammatory agent was confirmed by the decrease in the size of the lesion in the test group than the control.

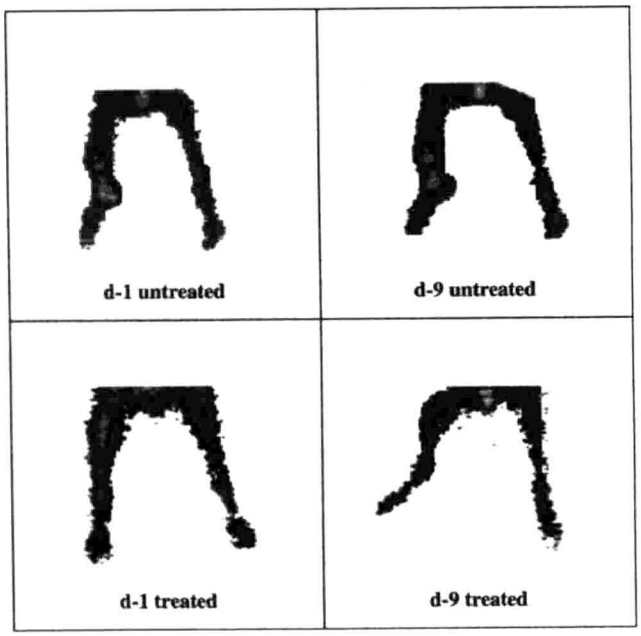

Fig. 2: Antiinflammatory action of *P. hexandrum*.

^{99m}Tc-dextran was injected intravenously in the rabbits bearing sterile inflammation in right thigh muscle. The scintigrams at day 1 and 9 of the treatment with the plant extract highlight the inflammed region indicting the drug's anti-inflammatory action.

Tunçay *et al.* in 2000, for the *in vivo* studies of microsphere formulations of diclofenac sodium (DS), used ^{99m}Tc labeled polyclonal human immunogammaglobulin (^{99m}Tc-HIG) as the radiopharmaceutical to demonstrate arthritic lesions by gamma scintigraphy[11]. After the induction of arthritis in knee joints of rabbits, the radiolabelled microspheres loaded with DS were injected directly into the articular cavity and at specific time intervals gamma scintigrams were obtained to find the residence time of the microspheres in knee joints in order to determine the most suitable formulation.

In the screening of various peripheral vasodilators, to be effective in frostbite, pharmacoscintigraphy has been used. ^{99m}Tc-MDP, a bone-scanning agent, has been used to study the effect of topical preparations containing agents like simrose oil, *Aloe soctrina* extract, $MgSO_4$, and minoxidil[12]. The percentage of the drug penetrated via skin was estimated from the vasodialation of cutaneous vessels. *Aloe soctrina* in water absorbable ointment base showed an increase of 10-15% in the vasodialation of cutaneous vessels. Simrose oil in a cream base ointment showed 10-15% increase in the vasodialation (fig. 3).

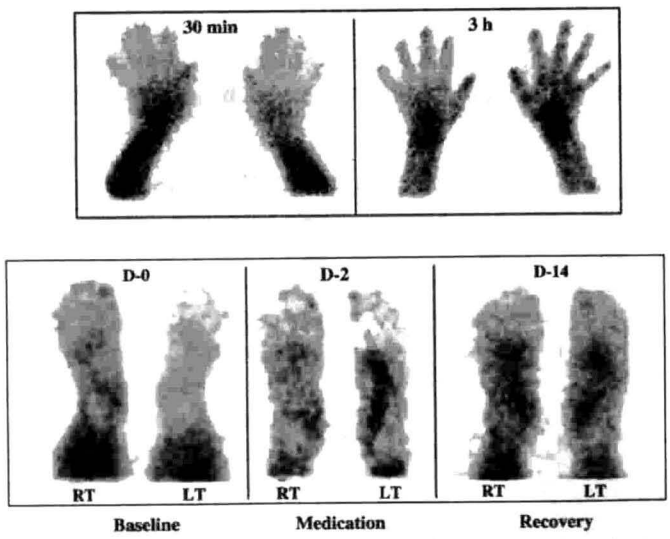

Fig. 3: Evaluation of various vasodilators for topical application (Simrose Oil).

Simrose oil in the cream base was applied to the extremities. Scintigrams taken before and after the use of topical creams showed increase in the vasculature.

PHARMACOSCINTIGRAPHY IN DRUG DELIVERY SYSTEM DEVELOPMENT

Modified release drug delivery:

A large fraction of researchers are working on the development of sustain release tablets/capsules. Pharmacoscintigraphy has been used for the *in vivo* evaluation of these formulations. Researchers have studied the exact site of the release, integrity of the formulation and percentage of drug released and then absorbed into the systemic circulation by this technique.

We carried out a study to evaluate the release pattern of cephalexin from a sustained release tablet using gamma scintigraphy. Cephalexin was radiolabeled with ^{99m}Tc and the labeled cephalexin was concentrated using a vacuum concentrator and the concentrate was dried in vacuum desiccator. Essential *in vitro* quality control parameters at acidic and basic pH were studied to assure the stability of the labeled cephalexin. The dried ^{99m}Tc-cephalexin was compressed with excipients to form a matrix tablet and a conventional tablet. These formulations were administered in humans and the gamma scintigraphs were obtained (fig.4).

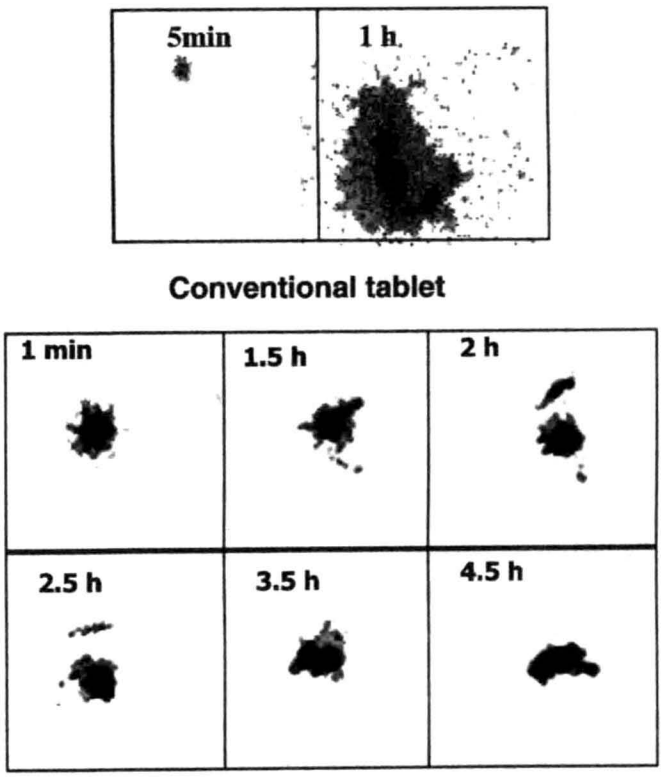

Fig. 4: Evaluation of conventional and sustained release tablet by pharmacosicintigraphy.

^{99m}Tc-cephalexin was compressed with excipients to form a sustained released matrix tablet and conventional release tablets. Scintigrams of the human volunteers at different time intervals after the ingestion of tablets show the release pattern in the GIT.

Colon drug delivery:

To increase the efficacy of any drug, the emphasis of the present R & D is on the delivery of the drug to a specific region of the gastrointestinal tract. For this purpose, colon and rectum are gaining importance as they help to reduce the first pass metabolism of many drugs. However, colon can be used as a site for peptide delivery. Pharmacoscintigraphy is probably the only technique, used to evaluate colon specific drug delivery systems *in vivo*. Scintigraphs obtained after administering a radiologically active delivery system can tell the exact site of drug release in gastrointestinal tract[13,14]. In a study carried out at Institute of Nuclear Medicine and Allied Sciences (INMAS), a colon specific capsule was studied in humans[15]. The ^{99m}Tc (free pertechnetate) was incorporated in the capsule by a pin puncture, which was sealed by a sealant. The sealed capsules were studied *in vitro* for any kind of leakage in acidic and basic pH. The *in vivo* images (fig. 5) of the capsules in humans were taken at the time intervals of 0.5, 1, 1.5, 3 and 5 h. The appearance of free pertechnetate in thyroid after 5 h confirmed the release of the drug in the colon, which corroborated with the *in vitro* dissolution profile of the capsule. No small intestinal release of activity was discovered.

Rectal drug delivery:

Rectal route of administration is the choice of route for many drugs. The biopharmaceutics of rectally administered suppositories developed by Jay *et al.* was studied, using labeled suppositories with radioactive marker ^{99m}Tc. In this study the degree of spreading of Witepsol H15 suppository in rectum was studied with ^{99m}Tc-hydroxymethyldiphosphate (HDP)[16]. Witepsol H15 suppository (2 g) was prepared containing 50 mCi of ^{99m}Tc-HDP. The scintigraphs, in males, obtained after self-administration of labeled suppositories were analyzed to generate activity versus time data for the interested area of rectum. Hay *et al.* used this technique where a steroid foam-enema labeled with ^{99m}Tc (pertechnetate) bound to an ion exchange resin has been used to study the spread of foam after rectal administration[17].

Pulmonary drug delivery:

The two methods of assessment currently available for inhaled drug delivery systems are; pharmacokinetic methods[18] and the technique of gamma scintigraphy. However, the latter process offered a significant advantage over pharmacokinetic methods in that it gives an indication of any differences in regional lung deposition[19]. The researchers, worldwide, have made the use of gamma scintigraphy as the most efficient method currently available for assessing the equivalence of inhaled medications. Scintigrams of the inhaled drug provide a valuable computerized image, which demonstrates the differences in total and regional lung deposition of inhaled medications. Gamma scintigraphy process shows a direct correlation between lung deposition and pharmacodynamic effect[21]. The deposition pattern in respiratory tract and oropharynx usually determines the efficacy of inhaled drugs. Pharmacokinetic parameters studied for any inhaled drug delivery system includes, lung capacity and forced expiration volume in first second (FEV1). Gamma scintigraphy technique involves the radiolabelling the drug, usually with technetium. The radiolabelling is validated, to ensure that it does not significantly change the particle size of the drug (which in itself would change the lung deposition) and the drug is delivered through the inhaler being studied (dry powder inhaler (DPI) and metered dose inhaler (MDI)). Immediately after inhalation the subjects are scanned using a gamma scintigraphy camera, which will clearly show the distribution of radiolabelled drug in the mouth, throat, lungs and stomach and permit quantification.

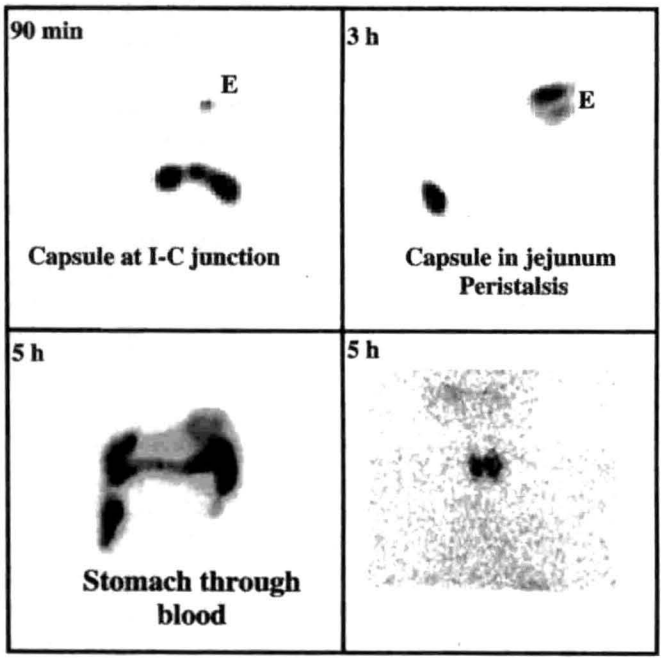

Fig 5: Pharmacoscintigraphic evaluation of colon drug delivery capsule.

$^{99m}TcO_4^-$ (free pertechnetate) was incorporated in the colon specific capsules by a pin-puncture. The scintigrams of human volunteers at different time intervals show the capsule released its contents at 5 h. This was confirmed by the appearance of stomach and thyroid as free pertechnetate accumulated in these regions.

Although MDIs have a fine particle fraction of 50% in the twin-impinge, these studies have shown that only 10 to 15% of the emitted dose actually was deposited in the lung, while the rest is nearly swallowed. As for DPIs, on the other hand, lung efficacy is more variable, ranging from 10% to as much as 30%. With the powder inhalers, lung deposition is more dependent on device design and construction.[20] A new analytical method has been developed by Pharmaceutical Profiles, Nottingham, a UK based company, to define regional lung deposition in a better way. The lungs are divided into a greater number of lung-shaped regions rather than using the traditional rectangular approach (http://www.pharmprofiles.com/gs3/radiolabelling.htm).

In a study carried out by Fok *et al.*, following delivery of ^{99m}Tc-labeled aerosols through a ventilator circuit, the amount of radioactivity in the lungs of 58 ventilated rabbits was estimated first by gamma scintigraphy via gamma camera and later by direct counting of the excised lungs (n=116 specimens) with a gamma counter[21,22].

Ocular drug delivery:

Pharmacoscintigraphy is a powerful tool to evaluate the ocular drug delivery systems. Precorneal retention and lacrimal clearance have been studied successfully by this technique. In a study carried out at Department of Pharmaceutical Sciences, University of Strathclyde by Wilson *et al.* gamma scintigraphy has been utilized to assess the precorneal residence of a radiolabelled polycarbophil formulation in healthy volunteers[24]. In order to radiolabel the formulation, they developed a novel method, which appears to be suitable for the general purpose labeling of polyacrylic ophthalmic gels.

In another study the ^{99m}Tc-DTPA was used to study the clearance of an ophthalmic formulation, containing mucoadhesive polysaccharide chitosan[25]. Gamma scintigraphic data showed that the clearance of the

formulation labeled with ^{99m}Tc-DTPA was significantly delayed in the presence of chitosan with respect to the commercial collyrium (TobrexÒ), regardless of the concentration and of the molecular weight of chitosan in solution.

Targeted drug delivery:

Nanoparticles and liposomes are the upcoming carriers for delivering the drug to target area[26]. But the need to estimate the goal achieved by designing such systems is a challenging area. Gamma scintigraphic technique can be exploited tactfully to trace these carriers *in vivo*. Nanoparticles/polymers/liposomes/microsheres[27]containing a particular drug can be studied by labeling the drug or the carrier with ^{99m}Tc. The biodistribution of chitosan nanopaticles with time in target and non-target organs was studied using serial Gamma Camera imaging (fig. 6). We have labeled nanocolloid/particles (sulphur, polysaccharides - PEG, dextran, hydroxyethyl starch etc) and liposomes and followed their biodistritubion non-invasively in humans. Efficacy of these drug formulations can be tested quantitatively directly in humans in a non-invasive and reproducible way[28].

PHARMACOSCINTIGRAPHY: INDUSTRIAL INTERESTS

The versatility of this technique is arousing the interest of pharmaceutical industry in India. Recently, a novel remote-controlled capsule system Enterion™ (Phaeton Research) has been developed, which may be opened at any point in the GIT by an external radiofrequency signal. Location of capsule in human intestine is tracked in real time by gamma scintigraphy (http://www.pharmprofiles.com/new.htm). The patented TechneCoat™ approach is a dry process, developed by Pharmaceutical Profiles and is highly applicable to the assessment of dry powder inhalers (http://news.moneycentral.msn.com/ticker/sigdev.asp?Symbol=GNBT). Intellisite(R) capsule is another radiofrequency activated, non-invasive disintegrating drug delivery device, developed by Scintipharma, Inc[30]. It is capable of non-invasive controlled delivery of drug formulations to the gastrointestinal tract for determining regional differences in drug absorption and bioavailibility. Radiolabelling permits determination of the capsule location within a specific region of the gastrointestinal tract via gamma scintigraphy. When the capsule reaches the desired site in the gastrointestinal tract, external activation opens a series of windows to the capsule drug reservoir. The release and degree of dispersion of the solution or powder contents from the capsule can be visualized.

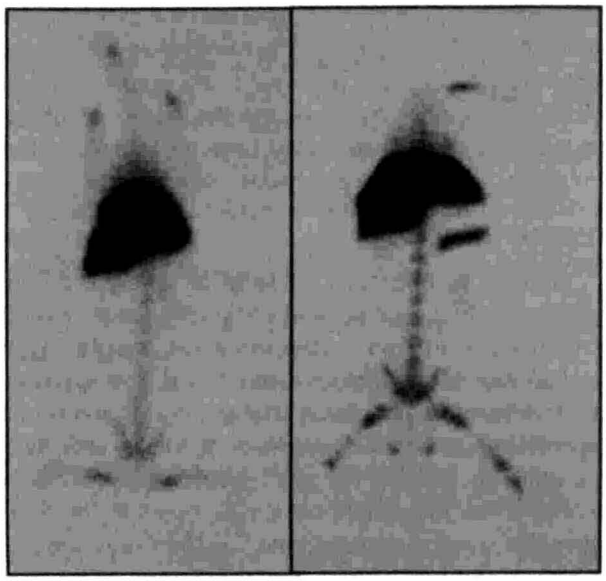

Fig. 6: Evaluation of ^{99m}Tc-chitosan nanoparticles.

Chitosan nanoparticles were radiolabelled with 99mTc by direct labeling method. Scintigrams of radiolabelled nanoparticles after intravenous administration into the rabbits shows the bones as targeted site.

Biotechnology Corporation, a leader in the area of buccal drug delivery, has carried out the gamma-scintigraphy studies of an Oralin™ formulation[29], the company's proprietary technology used to deliver oral insulin to show that that there is no lung deposition associated with buccal administration. The studies were conducted for the company by Pharmaceutical Profiles, Ltd. of the United Kingdom. The studies were undertaken to establish quantitative mouth deposition and the area of deposition for an Oralin spray formulation. The studies illustrated that the droplet size distribution of the formulation was too large to enter the deep lung region, and that the deposition of the formulation was located in the mouth, oropharynx, and the GI areas. There was no deposition of the formulation in the lungs. Prior to the *in vivo* study, *in vitro* experiments with a radiolabelled Oralin formulation were performed using an artificial lung simulator (Anderson 8-stage Cascade Impactor). The *in vitro* study was performed to determine the size distribution of the droplets of the Oralin formulation. These experiments suggested that the droplets of the Oralin spray formulation were too large (greater than 7 μ) to enter the deep lung regions. The positive *in vitro* experiments were followed by the *in vivo* human study. Seven healthy volunteers were administered the radiolabeled ^{99m}Tc Oralin spray formulation. Immediately after administration of the radiolabeled Oralin

formulation, subjects were imaged with the gamma camera to quantify the distribution of the formulation in the mouth, oropharynx, esophagus, stomach, and finally in the lung regions. Deposition was located in the mouth, oropharynx, and the GI areas. As expected from the results of the *in vitro* study, no lung deposition was observed.

CONSIDERATIONS

Gamma scintigraphy in spite of being a potential technique in drug development and pharmaceutical dosage form evaluation has some intricate problems. The radiolabelling of the molecule of interest in a formulation may limit the utility of gamma scintigraphy. The *in vitro* and *in vivo* stability of the radiocomplexed agent has to be studied thoroughly to explain the scintigraphs with confidence. Majority of the radiopharmaceuticals exists in the form of hydrophilic, polar molecules, and therefore provides model for water-soluble drugs. For the study of oil-based formulations containing hydrophilic drugs alternative models and techniques must be considered. Formulation procedure for any drug delivery device must be thoroughly studied. Effect of physical stress of vigorous processes like mixing, stirring, compressing, heating, etc. involved in the formulation procedure has to be considered before opting for this technique for evaluation. Chemical compatibility of the radiolabeled agent with other constituents must be assessed. Since these radionuclides, when tagged with the drug molecule, leads to a new compound, therefore to have a true picture, the radioactive agent should be confirmed to be a true tracer.

PHARMACOSCINTIGRAPHY: FUTURE

Positron Emission Tomography (PET) is an imaging modality, which can determine biochemical and physiological processes *in vivo* in a quantitative way by using radiopharmaceuticals labeled with positron emitting radionuclides as ^{11}C, ^{13}N, ^{15}O and ^{18}F and by measuring the annihilation radiation using a coincidence technique. Used in combination with suitably radiolabeled tracers, PET offers unique possibilities to investigate physiology, metabolism, pharmacokinetics, and pharmacodynamics and modes of action of drugs noninvasively in the intact human body, even pre-Phase-I. For *in vivo* pharmacokinetic studies of drugs or for gaining insight into the mode of action of drugs it is important, if not essential, to use a radiolabeled tracer with exactly the same properties as the parent drug[30-32].

Especially valuable is the contribution of PET to bridge the gap between molecular biology, understanding of pathology and to the design of a new generation of drugs. PET measurements can be performed after any route of drug administration, intravenous, inhalation or oral, however, intravenously administered drugs have been the most extensively evaluated. Functional MRI or fMRI is a recently developed technique to measure a derived parameter of the cerebral blood flow. Although PET is the best technique and in fact, the gold standard for measuring the rCBF using oxygen-15 labeled water as a diffusible tracer, fMRI uses the signal of hemodynamic changes using blood as an endogenous contrast agent as a measure for the blood flow. Although the direct measurement with PET has quite a number of advantages, such as sensitivity and total brain measurement, the non-radioactive measurement with fMRI has the advantage that a frequent repetition of the experiment is possible. Functional magnetic resonance imaging is extremely attractive as three-dimensional reconstruction of anatomical details allows the precise definition of the deposition and combined with scintigraphy, allows exquisite definition of site of deposition.

CONCLUSIONS

The use of imaging techniques particularly gamma scintigraphy to follow the behavior of drug formulations has revolutionized our knowledge of absorption and distribution in drug delivery. The development of gamma camera techniques as physiological tools to explore organ function became routine by the mid-seventies. Several research groups started to explore the applications of technique in drug delivery. Within 5 y, the utility of the technique became obvious and scintigraphy is now widely accepted as an important investigation tool in formulation research. Gamma scintigraphy is especially useful in exploring sources of inter-subject variation, especially in examining food effects in pharmacokinetic estimations and establishing windows of absorption for oral delivery. As a tool to examine drug delivery to the lung and to the eye, scintigraphy is the method of choice. Gamma scintigraphy is the technique of today, which needs to be exploited to the maximum for its potentials in the evaluation of new molecular entities, drug delivery systems/formulations and in therapeutic drug monitoring. PET and fMRI are the steps ahead of pharmacoscintigraphy and are the techniques of tomorrow. These techniques overcome the limitations of pharmacoscintigraphy and will be discussed in details in the Part-II of this review.

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