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Secondary X-ray fluorescence for in vivo transdermal absorption measurements

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Abstract

A modification of an X-ray fluorescence (XRF) technique that could be applied to the noninvasive measurement of transdermal absorption in human subjects with a low absorbed radiation dose was investigated. Secondary X-rays that were produced in a samarium foil by an isotopic ^{241}Am source were used to induce iodine K_α X-rays in an iodine-containing compound. The disappearance of this compound was measured following topical administration to a human volunteer. The use of the secondary X-ray fluorescence technique resulted in an 11-fold reduction in the absorbed radiation dose to the skin compared to conventional XRF bringing the technique within acceptable dosimetry limits for human studies.

Keywords: 5-Chloro-7-iodo-8-hydroxyquinoline; Noninvasive; In vivo percutaneous absorption; Topical delivery

1. Introduction

A number of methods have been used to measure the transdermal absorption of various drug compounds. These have included the application of radiolabelled compounds to the skin with subsequent measurement of radioactivity in blood or urine (Bartek et al., 1972), and the skin stripping technique where drug concentration in the stratum corneum after a specific period of time is correlated to the absorption rate of the drug (Rougier et al., 1986). An innovative approach involving attenuated total reflectance infrared spectroscopy for in vivo percutaneous enhancement measurements has also been reported (Higo et al., 1993). We have recently described the use of X-ray fluorescence (XRF) as a tool for the noninvasive measurement of percutaneous absorption by the surface disappearance method, and have validated the technique by comparing the results with those obtained by direct measurement of the absorption of a radiolabelled compound (Robert-

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son et al., 1992). These initial studies involved the application of a solution of 5-iodouracil to the skin and subsequently exposing the absorption site to an external ^{241}Am radioisotopic source. The 60-keV gamma rays emitted by ^{241}Am induced the emission of iodine X-rays from the application site. These X-rays, the number of which were proportional to the concentration of drug at the application site, were detected by a solid-state detector that was interfaced with a computer. We have also employed X-ray fluorescence to measure the effect of various transdermal enhancement vehicles on percutaneous absorption (MacLean et al., 1992) as well as the controlled release of a protein from a polymeric formulation (MacLean et al., 1995).

The above studies were all carried out in rats. For XRF to find utility in transdermal absorption measurements in humans, a critical consideration is the absorbed radiation dose to the application site resulting from exposure to the ^{241}Am source. Dosimetric measurements indicated that, at the source to skin distances we were employing (2 cm), the skin would receive an absorbed radiation dose of 540 mrem for each 5-min exposure normally used during our measurements. This is unacceptably high for use in humans. In order to reduce the absorbed radiation dose to the skin, an excitation photon that is closer in energy to the K-edge of the reporter atom iodine (33 keV) was required to maximize the fluorescence yield by increasing the photoelectric cross section. In addition, there was a need to attenuate the low-energy (17 keV) neptunium (Np) X-rays emitted from the ^{241}Am source that contribute a great deal to the absorbed radiation dose, but do not have enough energy to fluoresce the iodine atoms. In this paper, we describe the technique of secondary X-ray fluorescence that allows for percutaneous absorption measurements in human subjects at acceptable dosimetry levels by improving iodine X-ray fluorescence yields and attenuating low-energy Np X-rays.

2. Methods

The system employed for acquiring secondary XRF spectra was similar to that previously described (Robertson et al., 1992) except that a 0.25-mm thick samarium (Sm) foil was placed between the ^{241}Am source and the compound to be analyzed (Fig. 1). A circular hole was cut out of the Sm foil in order to allow the X-rays produced in the sample to reach the detector. The interaction of the 60-keV gamma rays emitted by ^{241}Am produced 40.1-keV X-rays in the Sm foil. These secondary Sm X-rays are capable of producing iodine K_α X-rays (28.6 keV) in compounds possessing an iodine atom native to their structure. The absorbed radiation dose that a human subject would experience from this system was measured using a thin-window ion chamber as well as exposing film badges to the source at a distance of 2 cm for a 1-h period.

Using this system, a standard sample containing 1.2 mg of 5-chloro-7-iodo-8-hydroxyquinoline (0.5 mg of I) was positioned 2 cm beneath the Sm

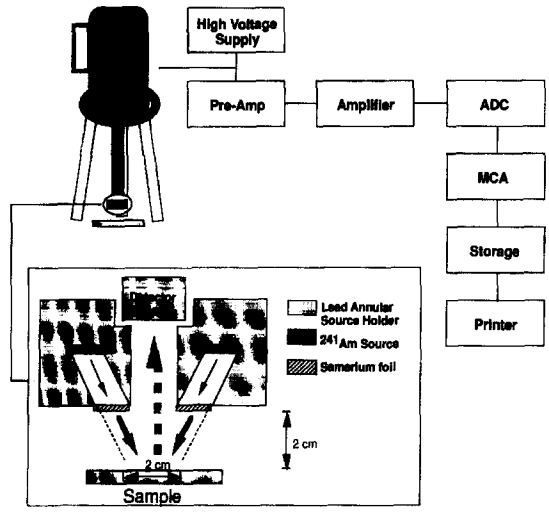

Fig. 1. X-Ray fluorescence system used for transdermal measurements in a human volunteer. The Sm foil was used to generate secondary X-rays that subsequently interacted with iodine atoms at the site of administration. Iodine X-rays were detected with a Si(Li) detector, and the amplified signal was processed with an analog-to-digital converter and a multichannel analyzer.

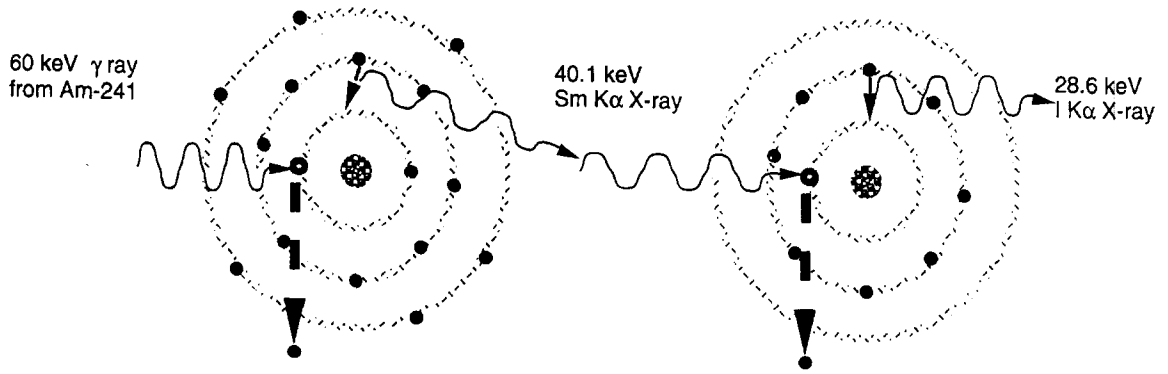

Fig. 2. Secondary X-ray fluorescence. Photons emitted by ^{241}Am interact with the Sm foil producing Sm X-rays. These subsequently interact with iodine in the active drug that was applied to the site of administration.

foil and was observed to yield 3.6 counts per second (counts/s). A 5-min background spectrum was then acquired in a human volunteer following informed consent as approved by the University of Kentucky Medical Institutional Review Board. A lead apron was worn by the volunteer to reduce exposure from scattered radiation. Secondary XRF spectra were then obtained immediately after the administration of a commercially available 3% cream preparation containing 5-chloro-7-iodo-8-hydroxyquinoline. Additional 5-min spectra were obtained at 2, 4 and 24 h following administration. Cotton gauze was taped over a plastic ring that had been placed around the site of administration to protect the site in between secondary XRF measurements. The concentration of drug at the application site at any one time was determined by integrating the area under the background-subtracted iodine K_α X-ray peak in the XRF spectrum. Previous studies determined that the disappearance of the iodine K_α X-ray signal from the site of administration over time is due primarily to the absorption and disappearance of the compound from the site of administration, and not to the attenuation of the signal by skin as the compound crosses the dermal barrier (Robertson et al., 1992).

3. Results and discussion

The 40.1-keV X-rays produced by the interaction of the 60-keV photons emitted by the ^{241}Am source with the Sm foil are much closer to the K-edge of iodine (33 keV; Fig. 2). The Sm foil also attenuated more than 90% of the low energy Np X-rays emanating from the source. The absorbed radiation dose from the secondary XRF setup which employed the samarium foil was measured to be 580 mrem/h. This is at least an 11-fold reduction in the absorbed dose observed from the standard XRF setup that did not exploit secondary X-rays from samarium. Therefore, the total absorbed dose to the site of administration of the volunteer using the secondary XRF setup was 242 mrem based on a total exposure time of 25 min (one background measurement and four absorption measurements). This is an acceptable skin dose and compares favorably to skin doses obtained from diagnostic computed tomography (CT) scans (500–700 mrem).

The disappearance of an iodinated compound from the site of administration following topical application to a human volunteer was measured by accumulating 5-min secondary XRF spectra at four different time points over a 24-h period. In Fig. 3, the number of iodine K_α X-rays accumulated is plotted as a function of time. After an apparent initially rapid absorption phase, essentially no change in the accumulated counts was observed after the second hour. The disappearance of the iodine K_α X-rays from the site of topical administration of an iodine-containing compound was previously confirmed using scinti-

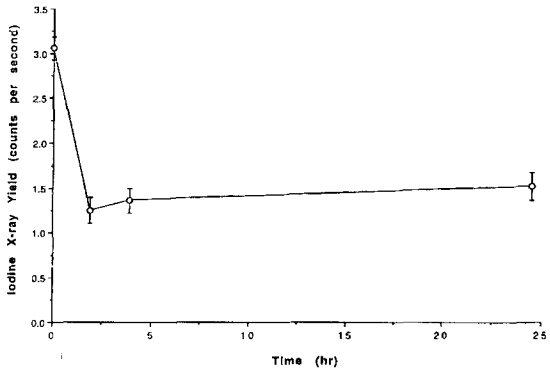

Fig. 3. Disappearance of a 3% 5-chloro-7-iodo-8-hydroxyquinoline cream formulation after topical administration to a human volunteer as measured by X-ray fluorescence.

graphic measurements (Robertson et al., 1992).

The secondary XRF approach has advantages over other in vivo transdermal absorption measurements in that, unlike the application of radioactive compounds where the entire body is potentially exposed to radiation, only the site of administration receives a small absorbed radiation dose. Thus, with secondary XRF, the absorbed radiation dose to internal organs is essentially zero; for ^{14}C-labelled compounds applied to the skin, the dose to internal organs can be hundreds of mrem, depending on how much activity accumulates in a specific organ and how long it is retained in that organ. Unlike the skin stripping method, multiple secondary XRF measurements can be made without altering the site of administration. This could be important when assessing drug concentrations in burned skin or at a wound site where skin stripping would be inappropriate.

XRF can be used to analyze a variety of elements, but is practically suited for elements with atomic numbers greater than 20. In general, the fluorescence yield can be expected to increase with increasing atomic number. XRF has been used in a variety of biomedical studies including the measurement of lead content in children's teeth (Bloch et al., 1976) as well as tissue mercury content (Walsh et al., 1973). However, halogenation with I or Br is readily achievable for a number of drug compounds, and XRF or secondary XRF can be employed to study their absorption following topical administration. The equipment required to carry out XRF experiments is modest in comparison to many other analytical instruments, is routinely used in radioanalytical laboratories, and is easy to operate.

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