Imaging Residue Transfer into Egg Yolks

Dan J. Donoghue*,† and Kyle Myers‡

Division of Animal Research, Center for Veterinary Medicine, U.S. Food and Drug Administration, Laurel, Maryland 20708, and Division of Electronics and Computer Science, Center for Devices and Radiological Health, U.S. Food and Drug Administration, 9200 Corporate Boulevard, Rockville, Maryland 20850

Prediction models for residue transfer into eggs are being developed. Recent results indicate that the developing egg yolk serves as an important storage depot for chemical residues. The current study was conducted to visualize incorporation and potential compartmentalization of drug residues in developing egg yolks. To this end, the drug magnevist was injected into hens to evaluate drug transfer into either early- or late-developing yolks. High-resolution magnetic resonance images (MRI) of drug residues in eggs were acquired using a 1.5 T Siemens Magnetom clinical scanner. A 10-cm circular surface coil was used for receiving the magnetic resonance signal. The eggs were positioned inside the coil cavity for an improved signal to noise ratio (SNR). Gradient-echo images were used to locate the centers of the eggs and to prescribe the position of the high-resolution image slab. The images were recorded using an inversion time (T1) weighted magnetization-prepared, rapid acquisition, gradient-recalled-echo (MPRAGE) pulse sequence. The sequence parameters used were as follows: repetition time (TR) equals 12 ms, echo time (TE) equals 5 ms, field of view (FOV) equals 200, TI = 10 ms, 1.25-mm slice thickness, and a matrix of 200 × 256. Following dosing, images of drug residues in eggs indicate that drugs can be incorporated and compartmentalized into ring structures within individual developing egg yolks. These results have significant human food safety implications because even after only a single dose, sequestered drug residues may be stored and later released to contaminate eggs for days to weeks after dosing.

Keywords: *Imaging; MRI; residues; yolk; food safety*

INTRODUCTION

Previously published results (Donoghue et al., 1996, 1997a) demonstrate that developing, preovulatory egg yolks (Figure 1) are an important storage depot for drug resides and other contaminants in laid eggs. Because of the unique physiology of the hen, even drugs with low lipophilic properties and short half-lives (30 min), such as ampicillin, can transfer and be stored in egg yolks for days to weeks prior to being laid (Donoghue et al., 1997b). This has significant human food safety implications because eggs may contain high levels of drug residues for an extended period even when drugs that usually do not create residue concerns in other edible tissues are used. This situation is unique for food-producing animals.

Fortunately, the pattern of residue transfer into developing egg yolk appears to be consistent for a 24 h exposure period for all chemicals tested to date (Donoghue et al., 1996, 1997a). This consistent pattern for preovulatory yolks was used to develop a working mathematical model to predict the pattern of residues in postovulatory, laid eggs.

The pattern of drug incorporation into developing yolks may be caused by a specific anatomical layering of yolk during development. It is known that egg yolk is not a homologous substance but is primarily formed by the daily deposition of yolky material in a ring pattern (Warren and Conrad, 1939). Somewhat like the growth rings in a tree, these yolk rings vary in diameter and can be visualized by staining or magnetic resonance imaging (MRI) (Grau, 1976; Hutchison et al., 1992). It has been speculated that the pattern of drug deposition is dependent upon, and proportional to, the amount of

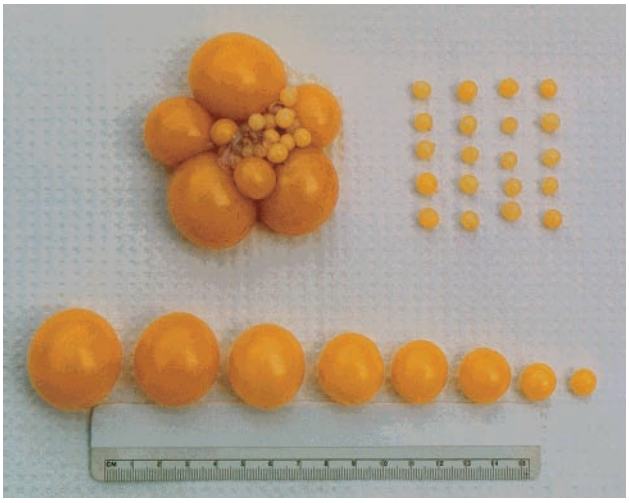

Figure 1. Photograph of an intact hen ovary (upper center) and dissected yolks from the large (lower) or small (upper right) phase of yellow yolk formation. Large preovulatory yolks (>0.2 g) are within 2 weeks of ovulation and arranged within a follicular hierarchy. The largest, heaviest yolk usually ovulates within 24 h, the second largest yolk usually ovulates 24 h after the largest yolk, the third largest yolk usually ovulates 24 h after the second largest yolk, etc. Small preovulatory yolks (<0.2 g) are within 2−6 weeks of ovulation.

* Address correspondence to this author at the University of Arkansas, Center of Excellence for Poultry Science, O-408 Poultry Science Center, Fayetteville, AR 72701 [telephone (501)575-2913; fax (501)575-7139; e-mail ddonogh@comp.uark.edu].

† Division of Animal Research.

‡ Division of Electronics and Computer Science.

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yolk material layered each day during yolk development (Donoghue et al., 1996). It is believed that yolks in the latter stages of development will incorporate drugs in the outer layers. Conversely, yolks earlier in development would include residues toward the inner layers of the growing yolk. Direct observation of these occurrences would provide strong support for the accuracy of the previously proposed egg yolk residue model.

Egg yolk rings have been visualized by MRI, so it is possible to visualize actual drug incorporation within these rings. Because traditional veterinary drugs would not be detectable by MRI, the human drug magnevist was used in this study. Magnevist is a contrast agent used in clinical medicine and would be expected to produce dark bands. This compound is a paramagnetic drug that develops a large magnetic moment in the presence of a magnetic field and is detectable under these conditions. Magnevist was injected into hens to evaluate drug transfer into either early- or late-developing yolks. Because our model predicts a universal pattern of residue transfer, the use of magnevist should produce results comparable to those of other veterinary drugs and result in visible rings when observed with MRI.

EXPERIMENTAL PROCEDURES

Single-comb White Leghorn hens, ~38 weeks of age, were used in this study. Hens were injected (iv) a single time on one day only or a single time on two consecutive days with 1.0 mL of magnevist ~1 h after oviposition. Dosing was done 1 h after oviposition to synchronize the time of dosing to yolk and follicular development. Because ovulation occurs within ~30 min after oviposition (Johnson, 1986), hens were dosed at the beginning of the daily phase of yolk accumulation. Hens were individually caged and had ad libitum access to standard laying hen feed and water and subjected to 14 h of light daily. Eggs were stored at 4 °C until subjected to MRI. The MRI was performed at Georgetown University Medical Center according to the procedure described by Hutchison et al. (1992).

Egg Residue Imaging. High-resolution MRI images of drug residues in eggs were acquired using a 1.5 T Siemens Magnetom clinical scanner. A 10-cm circular surface coil was used for receiving the MR signal. The eggs were positioned inside the coil cavity for improved signal to noise ration (SNR). Gradient-echo images were used to locate the center of the eggs and used to prescribe the position of the high-resolution image slab. The images were recorded using a T1-weighted magnetization-prepared, rapid acquisition, gradient-recalled-echo (MPRAGE) pulse sequence. The sequence parameters used were as follows: repetition time (TR) = 12 ms, echo time (TE) = 5 ms, field of view (FOV) = 200, inversion time (TI) = 10 ms, 1.25-mm slice thickness, and a matrix of 200 × 256.

RESULTS AND DISCUSSION

To understand drug incorporation into egg yolks, it is important to understand the fundamentals of egg yolk formation. Individual egg yolks develop over many months, with the latter phase of rapid yellow yolk accumulation occurring ~2 weeks before ovulation (yolks > 0.2 g; Griffin et al., 1984; Johnson, 1986; Burley and Vadehra, 1989). Additionally, there are many smaller yellow yolks (<0.2 g) waiting to enter the rapid growth phase that is ~2−6 weeks from ovulation (Griffin et al., 1984; Burley and Vadehra, 1989). These stages of yolk formation are depicted in Figure 1. During the 2-week phase of rapid yolk development before ovulation, there is a direct temporal relationship between the size of the developing yolks and their maturity and sequence of ovulation and incorporation into laid eggs (Griffin et al., 1984; Burley and Vadehra, 1989). As the larger yolks are ovulated, the smaller yolks are recruited to enter the 2-week period of rapid growth before ovulation (Griffin et al., 1984; Burley and Vadehra, 1989). Furthermore, during the period of large yellow yolk formation, daily yolk rings can be visualized by staining or MRI. Similar to the growth rings in a tree, the newest and largest rings are formed daily on the outside of the yolk.

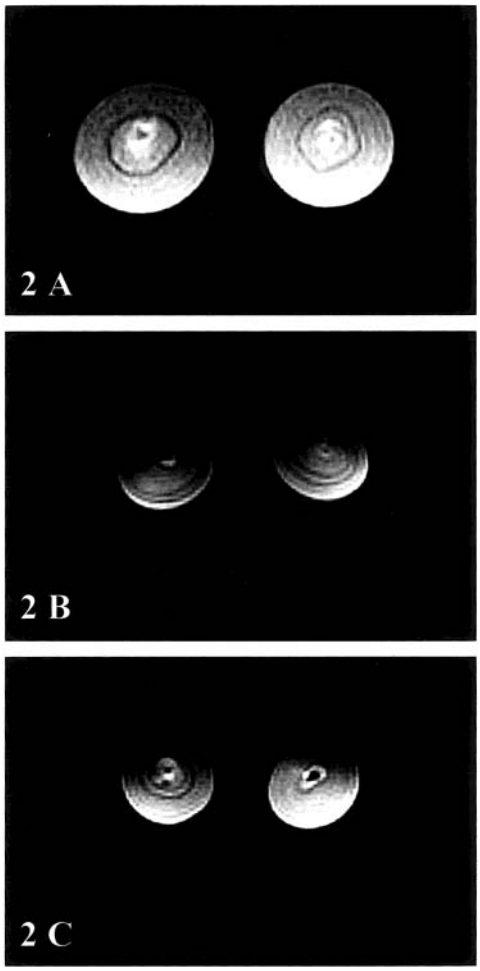

Figure 2. (A) Eggs collected 5 days from two different hens (left or right image, respectively) after a single injection of magnevist. Notice a single ring incorporates drug residues. (B) Eggs collected 3 or 4 days (left or right image, respectively) after single injections of magnevist on two consecutive days. Notice two separate rings incorporate drug residues. (C) Eggs collected 5 or 6 days (left or right image, respectively) after single injections of magnevist on two consecutive days. Notice two separate rings incorporate drug residues.

For the chemicals we have tested to date, the shape of the residue uptake curve for developing yolks during a 24-h period is consistent for a variety of drugs and other contaminants (Donoghue et al., 1996, 1997a). This assumption of similarity of an incorporation pattern for all potential contaminants is used as the foundation for our prediction model. However, traditional chemical and biological assays are not able to determine the location of residues within the developing egg yolk structure. To this end, whole eggs were imaged to determine if chemical residues are sequestered into individual rings within the developing egg yolks.

Our results indicate that dosing hens with the contrast drug, magnevist, produced dark bands on the internal rings of the egg yolks during development (Figure 2). Figure 2A represents eggs from a hen

injected just once with only one ring darkened, and panels B and C of Figure represent eggs from another hen injected on two consecutive days (two dark rings). In Figure 2A, the left and right eggs were collected 5 days after injection from two different hens. In Figure 2B, the left and right eggs were collected 3 and 4 days after the second injection, respectively. In Figure 2C, the left and right eggs were collected 5 and 6 days after the last injection, respectively. These images demonstrate that incorporation of drug residues into the ring structure is temporally related to the time of dosing. In other words, an egg laid soon after dosing has drug residues in the outer (younger) rings of yolks, whereas an egg laid later after dosing has residues in the inner (older) rings formed earlier in yolk development. These images support the concept that drugs can be incorporated and sequestered into developing egg yolks (Donoghue et al., 1996, 1997a,b).

This unique ability of laying hens to store drug residues in developing, preovulatory egg yolks has significant human food safety implication. Even ultrashort half-life drugs, which are quickly excreted from other body compartments, would still be stored in developing egg yolks. Thus, dosing laying hens, even with rapidly excreted drugs, could potentially expose the consumer to eggs containing drug residues for an extensive time period.

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