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Chorioallantoic membrane assays have been based on diffusion control—Problems arising with a diversity of mass transfers in egg white

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ABSTRACT

The chorioallantoic membrane (CAM) assays have been intensively used to determine angiogenesis and anti-angiogenesis of medicines. In view of bioactivity, this technique should be performed with kinetic control regime in chicken embryos. Whether the dosages ever used had satisfied this requirement, we explored by mathematical analysis. A diffusion-in-egg model was established to describe several medicinal diffusions in egg white that involved the instantaneous transient kinetic behavior, the diffusion of medicines in capping volume (the volume from the air sac to the interface of egg yolk). By reviewing the diffusion of various compounds including the cited and the experimentals in this work, we conclude that all the CAM assays ever cited were performed under diffusion control regime rather than kinetic control, which may bring forth deviations caused by a diversity of constitutes in egg white through various medicine–protein interactions.

1. Introduction

Deficiencies in oxygenation are widespread in solid tumors. The transcription factor hypoxia-inducible factor (HIF)-1α is an important mediator of the hypoxic response of tumor cells and controls the up-regulation of a number of factors important for solid tumor expansion, including the angiogenic factor, vascular endothelial growth factor (VEGF) [1].

Over the last 15 years, considerable progress has been made in the development of therapies based on targeting tumor angiogenesis [2,3]. However, although the induction of the hypoxia inducible factor 1α (HIF-1α) had been confirmed to be a positive factor for solid tumorigenesis, evidences indicate that it is not absolutely related to its regulation of VEGF expression [1]. In a study of two cell lines nullizygous for HIF-1α, one from embryos genetically null for HIF-1α, and the other from embryos carrying loxP-flanked alleles of the gene, which allows for pre-mediated excision, Ryan et al. [1] showed that the loss of HIF-1α negatively affects tumor growth in these two sets of H-*ras*-transformed cell lines, and this negative effect is not due to deficient vascularization. Despite differences in VEGF expression, vascular density is similar in wild-type and HIF-1α-null tumors.

Up to present, a huge number of documents had performed anti-angiogenic test with chorioallantoic membrane assay (CAM) [4–12]. Because of the presence of great variation in composition in egg white [13–15] and the diversity of chemical structures and polarity of the medicines tested [16], the question arises with "Can CAM accurately reflect the inherent response of a biological system to therapeutics with respect to angiogenic status?"

Egg composition varies with genetic selection and feedstocks. Egg Haugh unit (HU) had been altered as a result of genetic selection or by feeding with vanadium (V) to hens. In both altered HU conditions, eggs with low HU values yielded significantly less water-insoluble ovomucin from the thick albumen than eggs with high HU values, whereas the yield of ovomucin from thin albumen

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did not differ. The amount of ovomucin differed between eggs with high or low HU values as a result of feeding V, but the composition of ovomucin differed in thick albumen was not affected [13]. In comparison, egg white from high HU-line had lower contents of total carbohydrate, sialic acids, hexosamines, and hexoses than genetic lines with low HU. Conversely, thick albumen, whole albumen, and ratio of thick to thin were significantly higher in high HU than low HU line [13]. Purified ovomucin was isolated as an insoluble glycoprotein complex from thick egg white [14]. A homogeneous glycoprotein found in chicken eggs, designated α-ovomucin (molecular weight 210 kD) contains much lower contents of *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose, *N*-acetylneuraminic acid and sulfate than β-ovomucin, except mannose [14]. In addition, species-specific compositional variation also exists [15]. Moreover, interaction or the chemicals tested may trigger some signal related mechanism when interacts with glycoproteins in chicken egg-envelope [17]. We suspected that the effective dosage and responsive time in all CAM could be deviated by such many factors, i.e. "Which is actually the true rate-limiting step in a CAM assay?" In this present study, we established a mathematical model and simultaneously performed diffusion studies in egg white using some known authentic coloring matters and herbal extract.

2. Materials and methods

2.1. Diffusion-in-egg-model

2.1.1. Egg characteristics

Fresh chicken eggs and day-3 fertilized chicken embryos were purchased from the local egg wholesale company. The average interior dimension of chicken eggs is shown in Fig. 1.

By referring to Figs. 1 and 2 and assuming that the distance of C_o to C_i is a membrane mimic, the diffusion-in-egg-model can be established according to Fick's First Diffusion Law as

$$\frac{dn}{dt} = \frac{DA(C_o - C_i)}{\ell} \quad (1)$$

where dn/dt is the rate of flow through a plan with cross sectional area of A perpendicular to the abscissa (x-direction) along the longitudinal axis of egg. D is diffusion coefficient, the term $C_o - C_i$ is the concentration gradient across the gap (membrane mimic), ℓ is

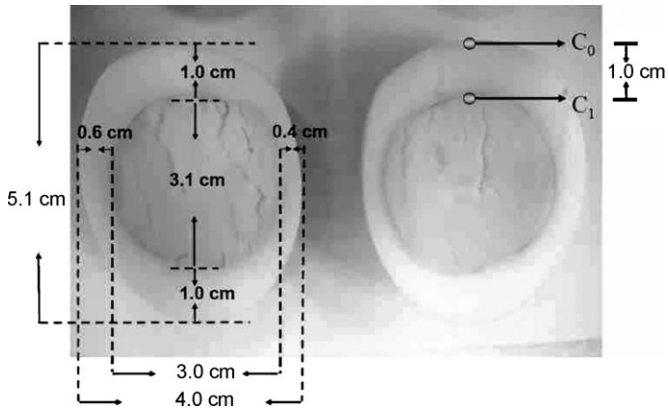

Fig. 1. General interior dimensions of chicken eggs. (Left) Chicken eggs appear as an oval shape, having at average a length of 5.1 cm in the longitudinal direction, 4.0 cm in radial direction. The egg yolk roughly has a diameter of 3.1 cm. (Right) the distance from the air sac to the interface of egg yolk is about 1 cm long. While this cross section part occupies a volume of 5 mL, about 10% of the volume of the egg with a total of 50 mL. The concentration of applied medicine at the initial position was designated as C_o, while that at the interface was C_i.

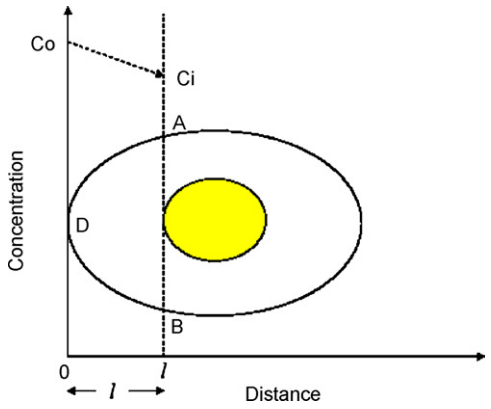

Fig. 2. Diffusion-in-egg model.

the thickness from the point D on the air sac to junction or interface of egg white (EW) and egg yolk (EY). The diffusion coefficient D is therefore calculable from the parameters including dn/dt, A, ℓ, and the concentration gradient $C_o - C_i$ (Eq. (1)). However the egg is oval in shape, the area A varies depending upon the thickness ℓ (Fig. 2); hence Eq. (1) is inapplicable at this moment. Recall that

$$A = r^2\pi \quad (2)$$

On differentiation of Eq. (2) we have

$$dA = 2\pi r\, dr \quad (3)$$

r is the radius at any point from C_o to C_i within the thickness ℓ. Alternatively, values of r are changing with thickness ℓ, i.e. the corresponding volume at certain distance of ℓ is

$$dV_{ABD} = dA\, d\ell \quad (4)$$

Substitution of Eq. (3) into Eq. (4) leads to

$$dV_{ABD} = 2\pi r\, dr\, d\ell \quad (5)$$

Integration of Eq. (5) yields

$$\int_0^v dV_{ABD} = 2\pi \int_0^r \int_0^\ell r\, dr\, d\ell \quad (6)$$

As indicated in Figs. 1 and 2, the total length of ℓ is 1 cm, thus the integral $\int_0^\ell d\ell = 1$ and Eq. (6) reduces to

$$\int_0^v dV_{ABD} = 2\pi \int_0^r r\, dr \quad (7)$$

Or

$$V_{ABD} = 2\pi \int_0^r r\, dr \quad (8)$$

where V_{ABD} is named hereafter as "the capping volume". In addition, we designate an additional terminology C_{inst}, which means the instantaneous concentration achievable in the capping volume V_{ABD}. C_{inst} can be attained provided the volume is very tiny, the egg white is very thin enough and homogeneously isotropic, more importantly the diffusion time should be very short. By definition

$$C_{inst} = \frac{Q_o}{V_{ABD}} \quad (9)$$

Or

$$C_{inst} = \frac{C_o V_a}{V_{ABD}} \quad (10)$$