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The LD50 Values of Cardiovascular Drugs at Different Developmental Stages in Chick Embryos

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Abstract

The LD50 values were determined for cardiovascular drugs in chick embryos at different developmental stages in order to obtain a more precise injection stage for fertile eggs of White Leghorn chickens for the prediction in rodents. First, time-course changes in the weight of fertile eggs, their air sac volume, and weight of each egg component were measured after the initiation of incubation. The weight of whole eggs decreased with incubation, while air sac volume increased. The chick embryos weight increased with decreases in albumen weight. These findings suggest that decreases in whole egg weight are due to decreases in water in the eggs. When the maximum volume of physiological saline or CMC-Na solution was injected into the air sac on different days of incubation, the bulk of the vehicle did not prove to be toxic to the chick embryos. Next, several cardiovascular drugs, i.e., aloprenolol, piretanide, dipyridamol, lidocaine, propranolol, canrenoate, disopyramide and reserpine were injected into the air sac of eggs on day 2, 5, 8, or 15 of incubation. Then, the chick embryos were sacrificed on day 20 of incubation, and LD50 values were calculated. The LD50 values of these drugs increased with the developmental stages in chick embryos. The LD50 values in chick embryos on day 2 and 5 of incubation showed a fairly strong correlation to those in mice obtained from the intravenous route. Furthermore, findings obtained on day 2 of incubation were most sensitive and reliable in predicting LD50 values in rodents. In conclusion, when cardiovascular drugs were injected into the air sac of fertile eggs in the early stage of development, i.e., day 2 of incubation, LD50 values in chick embryos could be used to predict the LD50 values in rodents.

Keywords: *cardiovascular drugs, chick embryo, developmental stage, lethal toxicity*

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Introduction

There has been criticism of the use of animals in animal experiments in recent years from an animal welfare viewpoint (Bruce 1985, Zbinden 1984). We focused on the use of chick embryos as an alternative to mammalian experiments and performed pharmacological and toxicological evaluations (Miyazaki et al. 1998, Sugiyama et al. 1996). Our previous study showed that LD50 values of cardiovascular drugs in chick embryos on day 2 of incubation accurately predicted those in rodents obtained from the intravenous route (Miyazaki et al. 1994). However, the injection day has been determined empirically, rather than based on scientific evidence, in teratogenicity studies using chick embryos. Therefore, we compared LD50 values obtained with chick embryos at different developmental stages with those obtained with mice in order to more precisely determine the optimal injection stage in the prediction of lethal doses in rodents.

Materials and Methods

Eggs and incubation

Fertile eggs of White Leghorns were obtained from Ohmiya Poultry Laboratory (Ohmiya) All the eggs were incubated at 37.6±0.2℃ at a relative humidity of about 65.5% and turned automatically every hour (Showa Incubator Laboratory, Urawa).

Test agent

The drugs tested were obtained from the following commercial sources; alprenolol HCl (Fujisawa), piretanide (Hoechst), dipyrydamol (Boehringer), lidocaine (Fujisawa), propranolol (Sumitomo), canrenoate potassium (Dainihon), disopiramide phosphate (Chugai) and reserpine (Daiichi). Drugs were diluted to the appropriate dosages with sterilized physiological saline.

Changes in egg component weight

The eggs were observed for weight on day 0, 2, 5, 8, 10 ,12, 15 and 18 of incubation by measuring whole egg, yolk, albumen, embryo and shell. Six fertile eggs per each incubation day were used.

Determination of the air sac volume

The maximum volumes of physiological saline into the air sac of fertile eggs were measured on day 0, 2, 5, 8, 10, 12, 15 or 18 of incubation. Six fertile eggs per each incubation day were used.

Lethal toxicity of vehicle

The fertile eggs were injected with a maximum volume of sterilized physiological saline or 0.5% CMC-Na solution on day 2, 5, 8, 10 or 15 of incubation, and they were measured for mortality on day 20 of incubation. Six fertile eggs per group were used.

Determination of LD50 values of drugs in chick embryos

Each cardiovascular drug was injected into the air sac of fertile eggs on day 2, 5, 8 or 15 of incubation. The viability of the embryos was checked daily by candling. The surviving chick embryos were sacrificed on day 20 of incubation and LD50 values were calculated by the Litchfield-Willcoxon method. The LD50 values were compared to those in mice from references (JPIC 1997, Sugiyama et al. 1992) . Six fertile eggs per group were used.

Statistical analysis

All LD50 values were transformed to natural logarithms. Then primary regression equations and correlation coefficients r were calculated using the least squares method. The linearity of primary regression lines were analyzed by one way ANOVA. The predicted LD50 values for each of the cardiovascular drugs used in mice were calculated using a primary regression line, and mean of the deviations (%) from the values of published refer-

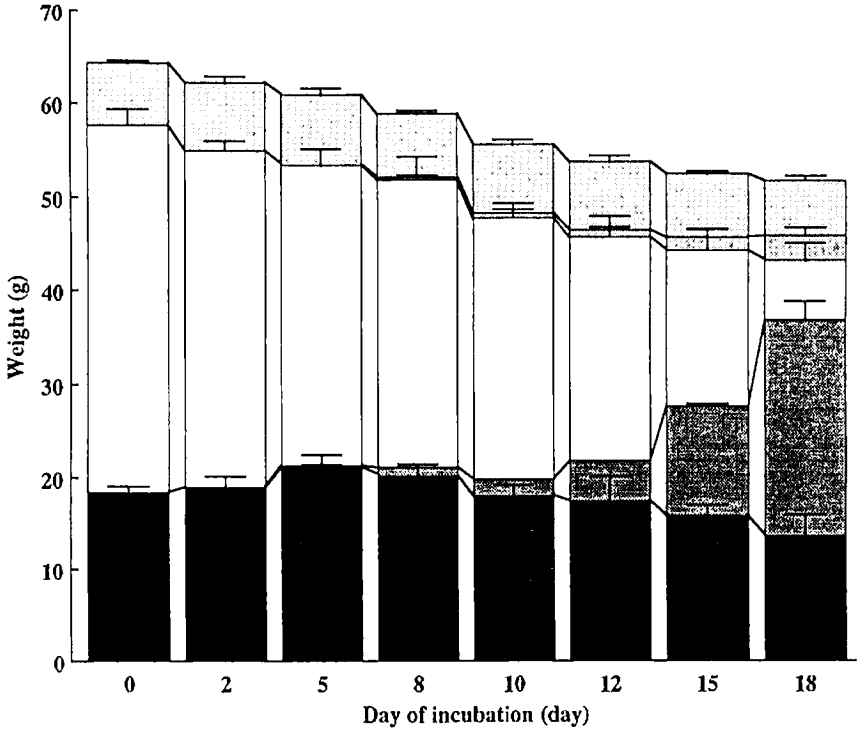

Fig. 1. Changes in the component of the fertile eggs through the incubation period. ▨, ▨, □, ▩ and ■ show shell, other, albumen, embryo and yolk, respectively. Data shown are the mean ± S.D. of 6 fertile eggs.

ences were defined as an accurate reference.

Results

Changes in egg component weight

The whole weight of eggs decreased consistently through the incubation period. The weight of each component in the eggs; shell, albumen and yolk decreased with the developmental stage. Most notably, there was a decrease in albumen. In contrast, embryonic weight increased (Fig. 1).

Determination of the air sac volume

The volume of physiological saline required to fill the air sac of fertile eggs increased with the developmental stages in the chick embryos. The weight of eggs decreased with the increase in the air sac volume by incubation day (Fig. 2). Consequently, a negative correlation between decreased egg weight and increased air sac volume was shown.

Lethal toxicity of vehicle

The maximum volume of physiological saline or 0.5% CMC-Na solution filled the space of the air sac at different days of incubation and no dead embryos were found. In other words, the bulk of the vehicle itself did not prove toxic to chick embryos regardless of the developmental stage.

LD50 values of drugs

The LD50 values of eight cardiovascular drugs showed linear increases with the developmental stages in chick embryos (Table 1). The LD50 values obtained with chick embryos and mice showed a strong correlation at each

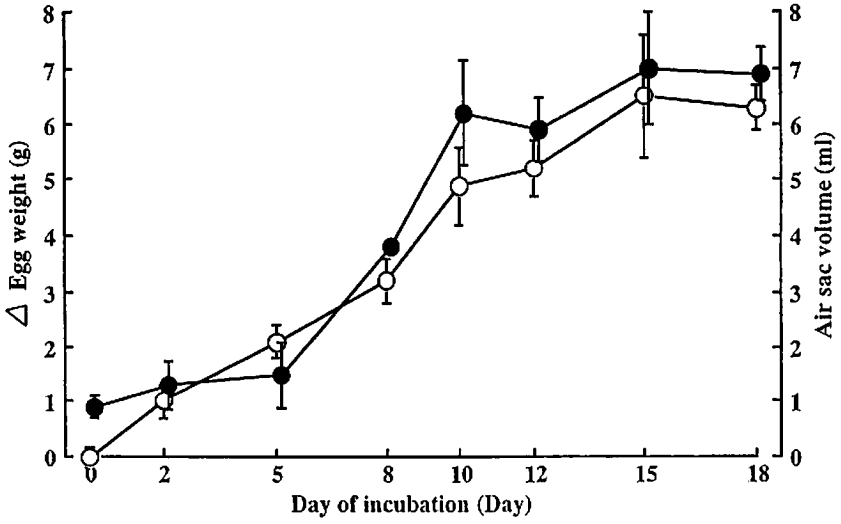

Fig. 2. Decreased whole egg weight (○) and increases in the volume of physiological saline to fill the air sac of fertile eggs (●). Data shown are the mean ± S.D. of 6 fertile eggs.

injection day. Especially, the LD50 values obtained on day 2 and 5 of incubation showed significant correlation with those in mice. When the LD50 values of chick embryos at different injection days were compared with those in mice, coefficients on day 2 of incubation showed a higher value than those of other injection days. (Table 2).

Discussion

The LD50 values of cardiovascular drugs in chick embryos at different developmental stages were determined in order to obtain a more precise injection stage in the chick embryo which will be valuable for prediction in rodents.

Unlike the mammalian, there is no time-lag between fertilization and the start of development in chicks; embryogenesis starts upon the initiation of incubation. Day 2 and 5 of incubation correspond to Hamburger and Hamilton Stages 12-17 and 25-28, respectively, and are equivalent to the organogenesis period. An effective heart beat and blood circulation have already become established by day 2 of incubation. By day 5 of incubation, the interventricuar septum shows a complete closure and the cardiovascular organogenesis is almost completed. The nervous system develops thereafter, and on day 8 of incubation, Stages 32-34, the neostriatum is formed. From day 10, embryogenesis is nearly completed and fetal growth begins thereafter (Butler 1987, Freeman 1967). Therefore, it should be possible to evaluate the lethal toxicity of cardiovascular drugs from day 2 of incubation. According to the reason described above, day 2, 5, 8 and 15 of incubation were selected to determine the LD50 values.

Whole egg weight decreased, while air sac volume increased each day after the initiation of incubation. The chick embryos weight increased while albumen weight rapidly decreased. These findings suggest that decreases in whole egg weight are due to decreases in water in the eggs. Injection of the maximum volume of vehicle into the air sac had no lethal effect on the chick embryos, suggesting that lethal toxicity can be evaluated not only by concentration adjustment, but by volume adjustment as well. This finding is thought to

Table 1.
LD50 values of cardiovascular drugs in chick embryos injected at different incubation times

Drugs	Chick embryos (μg/egg)				Mice[b]
	2	5	8	15[a]	(mg/kg)
Alprenolol	24	430	980	2850	29.0
Piretanide	300	2200	3850	6230	620.0
Dipyridamole	59	730	1100	1920	150.0
Lidocaine	13	210	380	1450	25.0
Propranolol	5.6	20.3	41.2	336	38.4
Canrenoate	40	490	2275	5354	87.0
Disopyramide	50	155	1414	2136	81.0
Reserpine	10	13.6	23.8	35.2	25.8

Drugs were injected into the air sac of eggs and observations were made on day 20 of incubation.
a) Day of incubation (Day)
b) LD50 values by the intravenous route were obtained from published references.

Table 2.
Regression equations, correlation coefficients and accuracy of LD50 values of cardiovascular drugs between chick embryos and mice at the different incubation days

Day of incubation	Regression equation	Coefficients "*r* "	Accuracy (%) [a]
2	Y=0.637+0.812X	0.914**	8.8
5	Y=0.783+0.455X	0.717*	31.3
8	Y=0.739+0.404X	0.677	30.4
15	Y=0.659+0.379X	0.582	36.7

Drugs were injected into the air sac of fertile eggs and observations were made on day 20 of incubation.
a) Accuracy (%) are mean of the deviations between the predicted LD50 values obtained from the regression equation and those in mice obtained from the intravenous route of published references.
*P<0.05, **P<0.01, significantly different by one way ANOVA.

be useful in the investigation of the lethal toxicity of poorly-soluble compounds.

The LD50 values obtained in the chick embryos showed linear increases with the incubation day. Similar phenomena were reported with non-cardiovascular drugs as well

and are thought to be attributable the fact that the necessary dose of drugs increases with increases in embryo weight (Schrankel et al. 1982, Sugiyama et al. 1986). Piretanide and canrenoate, both diuretics, showed the largest increases in LD50 values with the incubation day, and reserpine, a compound that acts on the central nervous system, showed the smallest increase and consistently showed lower values. These increases in LD50 values with time were lower than increases in embryo weight, indicating that drug sensitivity per weight of embryo increases as the developmental stage progresses. This means that the primordia of the target tissues for each drug already respond to its pharmacological effects at the organogenesis stage. Detailed pharmacokinetic actions of these cardiovascular drugs have not yet been investigated in chick embryos. Although, further investigation is necessary to clarify the pharmacokinetic actions for cardiovascular drugs in chick embryos.

The gradients of the primary regression line on day 2 of incubation was steeper than on the other days, suggesting a high sensitivity in whole egg. When a sufficient amount of the chemical agent was not obtained at the early stage of drug development, day 2 of incubation was the best day for drug injection in which to determine the LD50 values. The "Y" intercepts on the primary regression line showed almost the same values at all developmental stages. This fact may indicate that there are some intrinsic differences between the chick embryo and mice.

These results suggest that, while it may be possible to predict the lethal toxicity of cardiovascular drugs in rodents at any developmental stage using chick embryos, an early stage of development, i.e., day 2 of incubation, is the best stage from both prediction and sensitivity viewpoints.

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