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Influence of the lighting schedule on the toxicity of doxorubicin in chick embryos

Yuji Yoshiyama[1], Takashi Sugiyama[2], Hiroyuki Miyazaki[2], Hideyo Shimada[2] and Shigehiro Ohdo[3]

[1] Department of Clinical Pharmacy, Kyoritsu College of Pharmacy, 1-5-30, Shibakoen, Minato-ku, Tokyo 105 Japan, [2] Department of Clinical Pharmacology, School of Pharmaceutical Sciences, Kitasato University, 9-1, Shirokane 5 Chome, Minato-ku, Tokyo 108 Japan. and [3]Department of Clinical Pharmacokinetics, Division of Pharmaceutical Sciences, Graduate School, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812 Japan

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ABSTRACT

In the present study, we evaluated the toxicities of doxorubicin (ADR) in chick embryos using electrocardiograms (ECGs). The influences of the lighting schedule on the toxicities of ADR were then studied in these embryos. Fertilized eggs of White Leghorns were incubated and investigated on two occasions under a constant-light condition and a constant-dark condition. ADR (125, 250, 500 and 1000 μg) was injected into the air sac of each fertilized egg on the 16th day of incubation. ECGs were recorded after the injection.

Under the constant-dark condition, the lowest dose of ADR showed the maximum increase in the heart rate (HR). The HR decreased relative to the dose of ADR. Arrhythmias were induced at the highest dose. In contrast,

Corresponding author. Yuji Yoshiyama, Ph.D., Department of Clinical Pharmacy, Kyoritsu College of Pharmacy, 1-5-30, Shibakoen, Minato-ku, Tokyo 105 Japan. Tel 03-5400-2667, Fax 03-3434-5343

no significant changes were observed with any doses except at 1000 μg under the constant-light condition. The effects of 1000 μg were similar to those under the constant-dark condition.

These findings indicate that the manipulation of the lighting schedule has a marked influence on the toxicity of ADR in chick embryos.

INTRODUCTION

With the recent concern with animal rights, experimental studies using mammals have been limited in number and methods. Based on social acceptance, the experimental studies using chick embryos have drawn attention. Chick embryos are extensively used in biological studies (Miyazaki, et al., 1994, Tazawa et al., 1991, Rajala et al., 1984, Paff et al., 1975). To develop an alternative to the use of animals, we have studied the use of electrocardiograms (ECGs) in developing chick embryos (Sugiyama et al., 1996).

Doxorubicin (ADR) is an anthracycline antibiotic, which is produced by the fungus *Streptomyces peucetius* var. *caesius*. ADR is effective against various solid tumors. The major toxicity of ADR is cardiomyopathy.

Furthermore, ADR expresses circadian-stage dependent toxicity (Mormont et al., 1989). We have previously reported that the pharmacological effect of theophylline was markedly influenced by the lighting schedule (Yoshiyama et al., 1995).

The present study evaluated the toxicities of ADR in chick embryos using ECGs. The influences of the lighting schedule on the toxicities of ADR were then studied in those embryos.

MATERIALS AND METHODS

Fertilized eggs of White Leghorns (Omiya Poultly Laboratory, Saitama, Japan) were divided into two groups: a constant-light condition group and a constant-dark condition group. These eggs were incubated at 37.5 °C at a relative humidity of about 65%.

Four small holes every 90 degrees on "the equator" and one small hole on "the south pole" were made on each fertilized egg by an electric drill and sealed by Paraffin (m.p. 60 °C). The specially designed electro-needles were

inserted into the appropriate holes of "the equator" and "the south pole". The two needles on "the equator" were used as a bipolar lead of the embryonic heart, and the needle on "the south pole" was used as a ground lead. These needles were connected to the electrocardiograph system (Nihon Koden AVB-21, Tokyo, Japan). ECGs were recorded as bipolar waves between two needles on a thermal array recorder with a paper speed of 25 mm/sec (Nihon Koden PTA-1100M, Tokyo, Japan).

Doxorubicin hydrochloride preparation (Adriacin Injection, Kyowa Hakko, Tokyo, Japan) was used for the treatment. ADR (125, 250, 500 and 1000 μg) was injected into the air sacs of each fertilized egg on the 16th day of incubation. ECGs were recorded 0 to 60 minutes after the drug injection under a light condition or dark condition, and heart rate (HR) was determined from R-R intervals. Changes in heart rate were expressed as mean-percent changes of the drug-treated groups to the matched control.

RESULTS

Under the constant-dark condition, the lowest dose of ADR showed the maximum increase in the HR value. The HR decreased relative to the dose of ADR. Arrhythmias were induced at the highest dose (Fig. 1).

In contrast, no significant changes were observed with any dose except the 1000 μg under the constant-light condition. The effects of 1000 μg were similar to those under the constant-dark condition, the HR decreased gradually until 40 min (Fig. 2).

In the non-drug state, the heart rate of the chick embryos was not significantly different between the light and dark conditions. Moreover, there were no significant differences in the growth of the chick embryos at each lighting schedule, and the body weights of the chick embryos gradually increased during incubation.

DISCUSSION

With the chick embryo being one of the extensively used experimental animals, our recording system for electrocardiograms using the chick embryo may be applied as an animal test alternative. The chick embryonic heart develops similarly to that of mice, rats, and humans and also has a similar atrioventricular system (Bulter et al., 1987). It has been reported that ADR

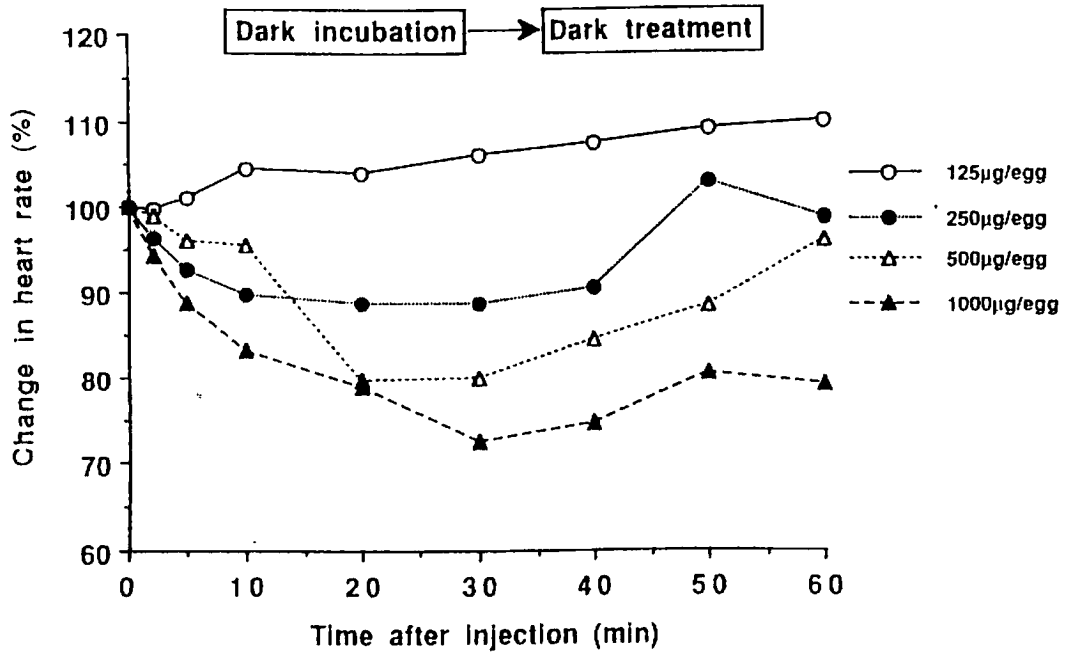

Figure 1. Changes in Heart Rate after Doxorubicin Injection at a Constant Dark Condition. Doxorubicin [125(-○-), 250 (-●-), 500 (-△-) and 1000(-▲-) μg] was injected into the air sacs of fertilized eggs on the 16th day of incubation. Changes in heart rate were expressed as mean-percent changes of the drug-treated groups to the matched control.

can induce damage to the cardiovascular systems (Zhang et al., 1994).

Circadian-dependent changes in pharmacological effects have been demonstrated for various drugs (Ohdo et al., 1996, Yoshiyama et al., 1996, Lin et al., 1994, Yoshiyama et al., 1993, 1992, 1989).

In the present study, the influence of the lighting schedule on the toxicity of ADR was demonstrated in chick embryos. Indeed, the lighting schedule modified the toxicity of the drug in the chick embryos. The ECG findings on ADR also corresponded well to the heart rates under the lighting schedule.

The lighting schedule appears to be an important factor in clarifying the circadian rhythm of drug actions. In mice housed under standardized light-

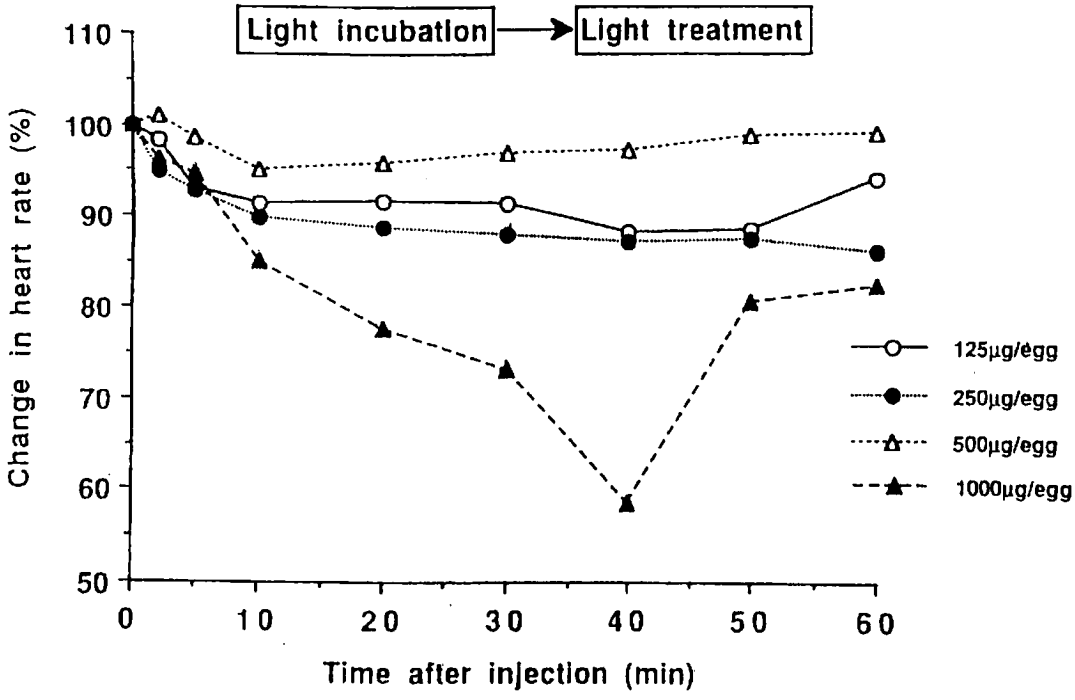

Fig. 2. Changes in Heart Rate after Doxorubicin Injection at a Constant-Light Condition. Conditions were the same as in Fig. 1.

dark cyclic conditions, there was highly significant circadian rhythm in mortality depending on the time of the drug administration. However, the circadian rhythm of the toxicity disappeared in mice housed in a constant-dark condition (Nakano et al., 1990).

The growth in chick embryos at each lighting incubation might change the development of the embryos. In the non-drug state, there were no significant differences in the growth or heart rates of the chick embryos both under the light condition and the dark condition. The body weights of the chick embryos gradually increased during incubation.

These findings indicate that the manipulation of the lighting schedule markedly influences the toxicity of ADR in chick embryos. In the chicken, the pineal body contains an endogenous oscillator and a phtoreceptor for the

circadian rhythmicity (Deguchi, 1979).

Although the exact mechanism underlying the influence of the lighting schedule on the toxicity of the drug remains to be clarified, lighting seems to enhance the toxicity of ADR in chick embryos.

CONCLUSION

Thus, the manipulation of the lighting schedule and the choice of timing for drug administration in relation to the lighting condition may help to achieve rational chronopharmacological studies of some drugs including ADR in certain experimental situations.

REFERENCE

Bulter, H., and Juurlink, B.H.J. (1987) "An atlas for staging mammalian and chick embryos" CRC Press, Inc.

Deguchi, T. (1979) A circadian oscillator in caltured cells of chicken pineal gland, Nature, 282, 94-98.

Lin, L., Grenier, L., Theriault, G., Grourde, P., Yoshiyama, Y., Bergeron, M.G., Labrecque, G., and Beachamp D. (1994) Nephrotoxicity of low dose of tobramycin in rats: effect of the time of administration, Life Sciences, 55, 169-177.

Miyazaki, H., Sugiyama, T., Saito, K., Kubota, N., Yoshiyama, Y., and Shimada, H. (1994) Toxicologic study of cardiovascular drugs in chick embryos and rodents, In Vitro Toxicol., 7, 243 -246.

Mormont, C., Boughattas, N., and Levi F.(1989) Mechanisms of circadian rhythms in the toxicity and efficacy of anticancer drugs, in "Chronopharmacology" ed. by B. Lemmer, pp.395-438, Marcel Dekker Inc.

Nakano, S., Song J., and Ogawa, N. (1990) Chronopharmacokinetics of gentamicin: comparison between man and mice, Annu. Rev. Chronopharmacol., 7, 277-280.

Ohdo, S., Watanabe, H., Ogawa, N., Yoshiyama, Y., and Sugiyama, T. (1996) Circadian rhythm of embryotoxicity induced by sodium valproate in mice, Eur. J. Pharmacol. Emviron. Toxicol. Pharmacol. Section, 293, 281-285.

Paff, G.H., and Boucek, R.J. (1975) Conal contribution to the electrocardiogram of chick embryo hearts, Anat. Rec., 182, 169-173 .

Rajala, G.M., Kuhlmann, R.S., and Kolesari, G.L. (1984) The role of dysrhythmic heart function during cardiovascular teratogenesis in epinephrine-treated chick embryos, Teratology, 30, 385 -392.

Sugiyama, T., Miyazaki, H., Saito, K., Shimada, H., and Miyamoto, K. (1996) Chick embryos as an alternative experimental animal for cardiovascular investigations: stable recording of electrocardiogram of chick embryos in ovo on the 16th day of incubation, Toxicol. Appl. Pharmacol., 138, 262-267.

Tazawa, H., Yamaguchi, S., Yamada, M., and Doi, K. (1991) Embryonic heart rate of the domestic fowl (gallus domesticus) in a quasiequilibeium state of altered ambient temperatures, Comp. Biochem. Phisiol., 101A, 103 -108.

Yoshiyama, Y., Kobayashi T., Ohdo, S., Ogawa, N., Bergeron, M.G., Labrecque, G., Beauchamp, D., and Nakano, S. (1996) Influence of a circadian-stage-dependent dosing schedule on the pharmacokinetics of isepamicin in humans, J. Infect. Chemother., 2: 106-109.

Yoshiyama, Y., Sugiyama, T., Kubota, N., Miyazaki, H., Saito, K., Tomonaga, F., Shimada, H., and Ohdo, S. (1995) Manipulation of lighting schedule can modify the pharmacological effects of theophylline in chick embryos, Biol. Pharm. Bull., 18, 776-778.

Yoshiyama, Y., Nishikawa, S., Sugiyama, T., Kobayashi, T., Shimada, H., Tomonaga, F., Ohdo, S., Ogawa, N., and Nakano, S. (1993) Influence of circadian-stage-dependent dosing schedule on nephrotoxicity and pharmacokinetics of isepamicin in rats, Antimicrob. Agents Chemother., 37, 2042-2043.

Yoshiyama, Y., Nakano, S., Ohdo, S., and Ogawa, N. (1992) Chronopharmacological study of valproic acid in mice: comparison of oral and rectal administration, J. Pharmacobio-Dyn., 15, 403-408.

Yoshiyama, Y., Nakano, S., and Ogawa, N. (1989) Chronopharmacokinetic study of valproic acid in man: comparison of oral and rectal administration, J. Clin. Pharmacol., 29, 1048-1052.

Zhang, R., and Diasio, R. (1994) Pharmacological basis for circadian pharmacodynomics, in: "Circadian Cancer Therapy" ed. by W.J.M. Hrushesky, 61-104, CRC Press Inc.