RADIOAUTOGRAPHIC STUDY OF THE POSTNATAL DEVELOPMENT OF THE PALATE FOLLOWING 5-FLUOROURACIL ADMINISTRATION IN MICE

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Ⅰ. INTRODUCTION

A number of investigators have been interested in the repression of the synthesis of normal nucleic acid by fluorinated pyrimidines. Since the synthesis of 5-fluorouracil, one of pyrimidine analogues, a series of experiments about its cytological effects have been made and it was found that 5-fluorouracil (5-FUR) is incorporated into ribonucleic acid (RNA) and inhibits the synthesis of deoxyribonucleic acid (DNA) by inhibiting methylation in thymine elaboration, and leads to defective protein synthesis.[1,2,3,4]

In working with higher forms of experimental animals, it has been shown that 5-FUR is taken up in greatest concentration by tissues where there are rapid cell multiplications. In high doses it is lethal and in lower doses it inhibits formation of new cells.[5] In alimentary organs, Levin and Martin[5,6] observed a decrease in mitotic activity of the rat small intestine following 5-FUR treatment and with respect to pancreas, they obtained the results in which the drug causes some interference with protein synthesis and also blocks the discharge mechanism of the cell. On the other hand, Stenram found a little change in rat liver with

the drug.[7] The administation of 5-FUR resulted in a sharp decrease in amylase content of the rat parotid gland as demonstrated by Kim and Han.[8]

Arvystas and Cohen[9] studied on the effects of 5-FUR on the secondary palatal development of mice and found that the drug appeared to interfere with the intrinsic force of shelf movements necessary for the midline fusion and thereby hinder the growth and development of the palatal tissue. On certain doses the drug has teratogenic activity in several species[10]

These studies suggest that the toxic side-effects induced by 5-fluorouracil are the greatest in tissues with a high metabolic activity. Nowadays, 5-FUR is one of the most commonly used chemotherapeutic agents in the treatment of human malignant tumors, namely carcinomas of gastrointestinal tract, ovary and breast. Inasmuch as the drug affects indiscriminatingly all actively growing cells, it is important that an understanding of its side effects be developed in relation to all metabolically active organs. Nevertheless, few studies in the past dealt with the effects of 5-FUR on young developing tissues.

The experiment reported in this paper was undertaken to study the effects of 5-FUR on the developing palate of newborn, normal and 5-FUR treated mice by means of radioautographic analysis of H^3-Thymidine (H^3-TDR) incorporation.

Ⅱ. MATERIALS AND METHODS.

A total of 20 neonatal mice of Balb/C strain were used. These mice were devided into two groups of 10 each; mice in the experimental group were given two daily intraperitoneal injections of 25mg/kg body weight of 5-FUR, while the control mice were given the similar injections of vehicle of physiologic saline alone.

The 20 experimental and control mice were injected with 5μCi/gm body weight of tritiated thymidine (specific activity 9.0 Ci/mM) intraperitoneally two hours prior to sacrifice. Two pairs of the experimental and control mice were sacrificed on days one, three, seven, fourteen, and twenty-one following the last injection of 5-FUR or vehicle.

Mice were sacrificed by decapitation and their heads were hemisected. The objects were fixed in 4% neutral buffered formalin for two days. Following fixation, the tissues were decalcified in 0.5M EDTA. They were double embedded and from them microscopic sections were prepared.

For radioautography, the slides were coated with Kodak NTB-3 nuclear track emulsion and processed in a routine manner. After 3 weeks of exposure in 4°C refrigerator, the slides were developed and stained with Hematoxylin and Eosin.

Ten selected sections from each mouse in each group were used to count the labeled cells present in the following areas; 1) the epithelium of the hard

palate, 2) the epithelium of the soft palate, 3) the periosteal osteoblast layer of the soft palate, and 4) the acinar cells of the glandular zone. Three or four black grains over a cell was the criterion for a labeled cell. The labeling index was determined by counting the number of labeled cells per 1,000 cells counted.[11-13]

Ⅲ. RESULTS.

Results are shown in Tables 1 through IV.

In all the areas studied the incorporation of H^3-TDR was generally suppressed. The time and the degree of suppression were variable; on day 7 the greatest effect was observed in the epithelium of the hard palate and the periosteal osteoblast layer but in the epithelium of the soft palate and the glandular zone it appeared on day 3.

From day 14 and on, there was a definite decrease of the inhibitory effects of 5-FUR in the epithelium of the hard palate and the periosteal osteoblast layer, whereas in the epithelium of the soft palate and the glandular zone it was from day 7.

By day 21, the incorporation of H^3-TDR was recovered in all experimental group and nearly stood up to the level of day 1.

Table 1. Labeling Indexes of the Epithelium of the Hard Palate

Day after 5-FUR Injection	Control (Mean±S.D.)	Experimental (Mean±S.D.)	Percent of Control
1	72.3±11.44	66.2±7.81	91.5
3	67.9± 7.05	55.5±8.86	81.7
7	60.4±10.74	48.1±8.23	79.6
14	49.4± 8.23	43.5±9.47	88.1
21	35.5± 7.53	31.4±8.08	88.5

Table 2. Labeling Indexes of the Epithelium of the Soft Palate

Day after 5-FUR Injection	Control (Mean±S.D.)	Experimental (Mean±S.D.)	Percent of Control
1	65.4± 6.19	61.0±9.59	93.3
3	63.8± 6.99	54.2±8.24	84.9
7	57.9±11.07	49.4±8.31	85.3
14	42.3± 8.61	38.0±9.97	89.8
21	36.0± 6.56	32.6±5.58	90.2

Table. 3. Labeling Indexes of the Periosteal Osteoblast Layer.

Day after 5-FUR Injection	Control (Mean±S.D.)	Experimental (Mean±S.D.)	Percent of Control
1	73.3± 7.67	62.9± 9.09	85.8
3	64.0±10.62	51.4±11.11	80.3
7	60.9± 9.86	45.4±10.49	74.4
14	41.5± 8.63	37.8± 7.59	91.1
21	31.2± 7.94	28.9± 6.32	92.6

Table. 4. Labeling Indexes of the Glandular Zone.

Day after 5-FUR Injection	Control (Mean±S.D.)	Experimental (Mean±S.D.)	Percent of Control
1	58.6± 6.49	50.4±8.93	86.0
3	50.8±11.34	40.8±7.39	80.3
7	49.6± 9.49	40.4±8.39	81.5
14	38.5± 7.49	33.3±6.23	86.5
21	31.5± 8.22	28.2±5.25	89.4

Ⅳ. DISCUSSION.

It has been demonstrated that 5-FUR has general toxic, cytotoxic, and subsequent teratogenic effects due to its mode of action.[1-5] However, clinical use of 5-FUR as a anticarcinogenic agent seems to be continually expanding. Malignant tumors of the young such as leukemia and certain melanomas call for the use of 5-FUR as a means of treatment for young patients.

Cronkite et al. stated that, although tritiated thymidine is apparently not a normal precursor of deoxyribonucleic acid, it can enter the synthetic chain and label DNA at the time of DNA doubling prior to mitosis.[13] Accordingly it is possible to follow cells from the time of DNA synthesis to ultimate death.

In the present study, the control mice revealed that there was a marked decrease in the labeling indexes of all the areas investigated during the period between the seventh and the fourteenth day. This is supported with the previous report for the postnatal development of the mouse palate by Griffiths et al.[14] They found that growth in width and thickness of the palate is slowest between the ages of five and ten days. For the control mice, the labeling indexes of both the hard and the soft palatal epithelium decreased with the passing of time, and reached to 35.5±7.53 in the hard palate and 36.0±6.56 in the soft palate by day 21. This agrees with Toto and others; their experiment indicated the labeling

index of 45.19±11.04 for the mice aged one month.[12]

During the experimental period the labeling indexes were gradually reduced. This trend in labeling index might be considered as the decreased mitotic activity resulted from the progress of growth.

Osteoblast layer of the hard palate is a major site that was highly affected by 5-FUR. Cho et al. indicated that growth of the skull as expressed by histomorphic differentiation of synchondroses may be significantly impaired during the early period of postnatal life under 5-FUR influence.[15] Choi's experiment demonstrated the reduction of the thickness of the epiphyseal cartilage in rat femur following 5-FUR administration.[16]

As well as the periosteal osteoblast layer, the acinar cells of the glandular zone were strongly affected in the early period of this experiment, but the rate of recovery from the influence of 5-FUR was slower in the acinar cells rather than osteoblasts. Studies by Kugler and others proved that the drug causes profound changes in the structure and function of the pancreas, a tissue with little cell replacement.[17] It was demonstrated by Kim that the administration of the agent represses the protein and nucleic synthesis in four digestive glands; parotid, submandibular, sublingual gland and pancreas.[18] Levin has insisted that the decrease in intestinal function induced by 5-FUR during the initial 3-day treatment appear to be caused mainly by reduction of cell numbers through suppression of crypt mitosis rather than by interference with functioning cells on the villi.[6]

According to Kim[18] in a radioautographic study, it should be remembered that the radioautographic grain numbers may not reflect the capacity of the cells to synthesize proteins and nucleic acids, but the rate of incorporation of labeled precursors in a given cell population.

V. SUMMARY AND CONCLUSIONS.

In order to examine the effects of 5-FUR on the postnatal development of the palate, twenty neonatal mice of Balb/C strain were selected and given two daily intraperitoneal injections of 25 mg/kg body weight of 5-FUR, or the similar injections of saline without the drug. The specimens were radioautographically studied in four palatal areas; 1) the epithelium of the hard palate, 2) the epithelium of the soft palate, 3) the acinar cells of the glandular zone, and 4) the periosteal osteoblast layer of the hard palate. Following results were obtained;

1. During the entire experimental period labeling indexes were gradually decreased.
2. On day 3, a considerable effect of 5-FUR was observed in the glandular zone and also the epithelium of the soft palate. On day 7, the periosteal osteoblast

layer and the epithelium of the hard palate were most strongly affected by the drug.

3. In the glandular zone and the epithelium of the soft palate, there was a decrease of the inhibitory effects of 5-FUR from day 7.
4. From day 14 and on, there was a definite decrease of inhibitory effects of 5-FUR in the epithelium of the hard palate and the periosteal osteoblast layer.
5. By day 21, the incorporation of H^3-TDR was recovered in all areas and nearly stood up to the level of day 1.
6. Among the four areas, the periosteal osteoblast layer was the most seriously affected area by the drug and the most rapidly recovered from the inhibitory action of 5-FUR.

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≫국문초록≪

5-Fluorouracil이 백서의 구개발육에 미치는 영향에 대한 자기방사법적 연구

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남 동 석

항암화학요법제인 5-FUR이 백서의 구개발육에 미치는 영향을 연구코자 20수의 신생백서를 반씩 나누어 실험군에는 체중 kg당 25mg의 동 약제를 2회 복강내 주사하고 대조군에는 생리적 식염수만을 주사한 후 제 1, 3, 7, 14 및 21일에 각각 2수씩 도살하였다. 또한 도살 2시간전에 체중 gm당 5μCi의 H^3-TDR을 주사하여 자기방사법적으로 처리 관찰하였다. 결과는 다음과 같다.

1. labeling index는 실험일자의 경과에 따라 점차 감소하였다.
2. 5-FUR의 억제작용이 골아세포층과 경구개의 상피층에서 제 7일에 가장 강하게 나타났고 제14일부터는 감소되는 경향을 나타내었다.
3. 연구개의상피층과 glandular zone에서는 5-FUR의 억제효과가 제 3일에 가장 심히 나타났으며 제 7일부터는 감소되었다.
4. 5-FUR의 억제효과는 점차 감소되어 제21일에는 대조군과의 차이가 현저히 감소하였다.
5. 골아세포층이 5-FUR의 영향을 가장 심하게 받는 반면에 그 회복도 가장 빨랐다.

EXPLANATION OF FIGURES

Fig. 1 The radioautograph of hard palate of control mouse showing silver grains scattered over basal layer of the epithelium and the perisoteal osteoblast layer on day 14 (×100).

Fig. 2 The radioautograph of experimental mouse compared with Fig. 1 (×100).

Fig. 3 Radioautographic grains over basal layer of the epithelium of hard palate on day 7, control (×400).

Fig. 4 The radioautograph of experimental mouse compared with Fig. 3 (×400).

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