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THE STEREOLOGICAL STUDY OF AGE–RELATED EFFECTS IN RAT THYROIDS AFTER γ–RAY IRRADIATION

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ABSTRACT

The infant (0.5 month) and adult rats (2.5, 6 and 15 months, respectively) were exposed to single γ–ray neck irradiation (0Gy, as control, 0.5Gy, 2Gy, 4Gy, 8Gy, 16Gy). Some morphometric parameters of thyroid gland were measured under light microscope at 6 weeks after exposure. The results showed that the infant rat thyroids changed significantly after 0.5Gy, whereas the adult thyroids expressed the similar response after more than 2Gy. Analysis of these data suggested that the infant thyroids were more radiosensitive than the adult ones.

Key words: radiation effect, γ–ray, thyroid, age–related.

INTRODUCTION

Thyroid is one of the important target organs in nuclear bomb explosion and reactor accident (ICRP,1984). Age is an unnegligible factor which can influence the organism's sensitivity to radiation (Book et al., 1980). Some epidemiological survey on medical exposure to human thyroid indicated that the incidence of hypothyroidism depended on dose and age of exposed individuals (Green et al., 1980; Tarbell et al., 1990). But other studies didn't support this conclusion (Devney et al., 1984). Some experimental studies on thyroid of animals based mostly on qualitative observation and unstable functional changes have shown that age related differences existed in radiation injury (Book et al., 1980; Sikov, 1969). Consistent view hasn't been given about age–related deterministic effects in thyroid so far.

MATERIAL AND METHODS

Twenty–four male Wistar rats in every group (0.5, 2.5, 6 and 15 months) were usually divided into six subgroups randomly. The infant rats were bred by their mothers before and after exposure. The neck area of infant (under normal condition) and adult rats (1% vinbarbital sodium intraperitoneal injection) were exposed by γ–ray(^{60}Co) with doses of 0.5, 2, 4, 8, 16 Gy and 0 Gy (as control). The irradiation field was 2×4 cm. The exposure rate was about 8.0×10^{-4}C / kg • sec.
All animals were sacrificed and their thyroid glands removed at 6 weeks after the expo-

sure. Samples of thyroid were fixed by immersion in Bouin's solution for 24h at 37℃, dehydrated gradiently by alcohol and xylol and embedded in paraffin. The 4μm thick sections were stained with hematoxylin and eosine.

Morphometric parameters of thyroid were measured with MIAS−300 image analysis system made by Sichuan University. The magnification was 650 times for the components of thyroid, and 3250 times for follicular epithelial cells. The direct measurement two−dimensional parameters were area, perimeter, equivalent diameter and form factor. The derived stereological parameters were volume density, equivalent diameter and 3D−form factor (Sheng & Shen,1991).

Data were analyzed by poly−way analysis of variance and stepwise regression analysis.

RESULTS

The quantitative studies showed dose and age having a significant influence on the structure of thyroid, the reciprocal effects between them were also significant for some morphometric parameters (Tab.1). The mean diameter and 3D−form factor of follicular cell nuclei changed significantly after exposure (Tab.2). The volume density of follicular lumen decreased along with dose increase. On the other hand, the volume density of the glandular interstitium increased after exposure (Tab.3). The volume density of thyroid

Table 1. The results of statistic analysis on morphometric parameters in rat thyroid

Parameter	Factor	Degree of freedom	F	P
	D	3	23.14	<0.01
Dq(N)	A	5	208.2	<0.01
	D×A	15	4.418	<0.01
	D	3	21.81	<0.01
F(N)	A	5	107.4	<0.01
	D×A	15	5.224	<0.01
	D	3	128.7	<0.01
Dq(f)	A	5	22.99	<0.01
	D×A	15	0.967	>0.05
	D	3	203.2	<0.01
Vv(f)	A	5	38.98	<0.01
	D×A	15	4.142	<0.01
	D	3	229.3	<0.01
Vv(p)	A	5	4.793	<0.01
	D×A	15	1.937	<0.05
	D	3	60.35	<0.01
Vv(i)	A	5	78.58	<0.01
	D×A	15	1.537	>0.05

D: dose; A: age; D×A: reciprocal effect
Dq(N): equivalent diameter of nuclei
Dq(f): equivalent diameter of follicular lumen
F(N): form factor of nuclei
Vv(f): volume density of follicular lumen
Vv(p): volume density of parenchyma
Vv(i): volume density of interstitium

parenchyma (age < 6month) had a hyperplastic change after no more than 4Gy. In conclusion, the infant thyroids changed significantly after 0.5Gy exposure, but the adult thyroids expressed similar response after about 2Gy.

Table 2. The equivalent diameter of thyroid epithelium nuclei in different-aged rats after γ-ray neck irradiation

Dose(Gy)	Dq(N)(μm)			
	0.5 month	2.5 month	6 month	15 month
0	4.88 ± 0.16	5.31 ± 0.10	5.34 ± 0.25	5.18 ± 0.48
0.5	4.27 ± 0.16***	5.05 ± 0.33	5.02 ± 0.17	4.74 ± 0.07**
2	4.14 ± 0.08***	4.64 ± 0.88***	4.74 ± 0.17	4.30 ± 0.12***
4	4.05 ± 0.07***	4.49 ± 0.14***	4.39 ± 0.07***	4.30 ± 0.07***
8	3.98 ± 0.09***	4.03 ± 0.09***	3.91 ± 0.10***	3.89 ± 0.07***
16	3.60 ± 0.21***	3.47 ± 0.06***	3.60 ± 0.20***	3.55 ± 0.19***

* $P<0.05$, ** $P<0.025$, *** $P<0.01$ ($\bar{X} \pm SD$, compare with control group)

Table 3. The volume density of interstitium of thyroid different-aged rats after γ-ray neck irradiation#

Dose(Gy)	Vv(i)(%)			
	0.5 month	2.5 month	6 month	15 month
0	1.7 ± 0.4	2.3 ± 0.4	3.2 ± 0.1	8.6 ± 0.8
0.5	3.5 ± 0.6***	2.6 ± 0.6	3.9 ± 0.8	8.8 ± 0.4
2	3.9 ± 0.2***	4.2 ± 0.7**	6.4 ± 0.2***	9.0 ± 0.7
4	4.3 ± 0.6***	5.7 ± 1.6**	8.9 ± 2.2***	9.6 ± 0.3
8	8.7 ± 1.1***	8.8 ± 1.9***	13.4 ± 1.5**	13.2 ± 1.8***
16	10.0 ± 1.0***	12.2 ± 3.7***	14.0 ± 1.2***	14.2 ± 1.1***

see also Tab.2.

The dose-effect and age-effect relationships were given by function equations. Cooperative effects have been seen in the change of Vv(p) and Vv(i). Instead, contradictory effects have been found in the changes of Dq(N), Dq(f) and Vv(f) (Tab.4).

Table 4. Quantitative relationships on morphometric parameters, dose and age in rat after γ-ray neck irradiation

Parameter	Functional equation*	R**
Dq(N)(μm)	$Dq(N) = 4.805 - 0.08816D + 0.002489A$	0.8650
Dq(f)(μm)	$Dq(f) = 36.18 - 0.4997D + 1.080A$	0.9594
Vv(f)(%)	$Vv(f) = 29.28 - 0.4592D + 0.9354A$	0.9021
Vv(p)(%)	$Vv(p) = 68.01 - 0.1117D - 1.251A$	0.9538
Vv(i)(%)	$Vv(i) = 2.660 + 0.5637D + 0.3535A$	0.9324

* D: Dose(Gy); A: Age(month); ** $P<0.05$

DISCUSSION

Most morphological studies on thyroid showed that the size and weight of thyroid increased with growing up. But when an adult became older, changes appeared including volume atrophy, fiber hyperplasia, follicular modifications, etc (Sidney, 1978). The size distribution of rat thyroid follicle became smaller gradually from periphery to central part of gland. The ratio of follicular lumen and interstitium increased, but the ratio of parenchyma decreased along with the age increase. These fine changes of thyroid structure generally didn't have influence on the normal function of thyroid.

Animal experimental studies have shown a very slow cell–renewal, a low mitotic rate, and a relative radioresistivity (Tubiana et al., 1990) in the normal adult thyroid. Malone et al. found the thyroid tolerance dose in the rat as approximately 18Gy gamma–radiation (Malone et al., 1974). Little histologic damage develops after doses of 2.5Gy or less in rat thyroids (Clifton,1986). However, some markedly morphologic subtle modifications were found in rat thyroids after small doses (infant rats 0.5Gy, adult 2Gy) in our study. The infant thyroids were more radiosensitive than the adult ones. The superficial site of the gland, the high mitotic rate and the prolific arterial supply probably determine the radiosensitivity in infant rats. More subtle modifications, which couldn't be found in the past, were observed because of the new method of measurement.

Cell killing is the main but not the only process involved in deterministic effects (ICRP,1990). The size and shape of epithelium nuclei changed markedly in infant thyroids after 0.5Gy in our study. Sloughed cells were observed after 4Gy. Similar modifications could be seen after 8Gy X–ray fractional neck exposure in rabbit (Alcaraz et al., 1990). Hyperplastic modifications were also observed in epithelium after no more than 4Gy. This phenomenon was due to the compensatory adjustment in organism. Because of powerful reservation in infant thyroids, this response were more obvious in infant rats than in adult ones. From this point, we can explain why the function of thyroid can maintain normal level of what for a long time after radiation injury. In our study, we found the interstitium of thyroid changed significantly after exposure. The results showed the interstitium of thyroid as sensitive to radiation. Numerous histological studies in the past have reported that vascular damage often plaied an important role in some serious late effects (Holten,1983). More serious fibrosis in infant thyroids perhaps resulted in higher incidence of thyroid cancer.

Cooperative and contradictory effects were seen in the change of some morphometric parameters. These changes were consistent with qualitative observations. Therefore, the conclusion is trustworthy in a certain dose and age range involved in our study.

In a word, the infant rat thyroids were more radiosensitive than the adult ones. Recently, data on atom bomb survivors in Japan and victims in Chernobyl nuclear accident showed that the injury of thyroid by ionizing radiation, particularly in fetal and infant thyroids, is a very serious problem which most people pay attention to. Thus, it is necessary and appropriate that the infants and children must be protected particularly in the nuclear accident for decreasing the harmful effect of radiation on thyroid.

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