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Age factor in post-nephrectomy compensatory renal growth

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Summary. In a retrospective investigation we found that the endogenous creatinine clearance (Ccr) of 35 nephrectomized patients was lower than that of normal people of comparable age with two intact kidneys. The Ccr level in the post-nephrectomy patients appeared to correlate with the age of the patients at the time of nephrectomy. The average Ccr value was 99.2 ml/min in the younger group, and 54.9 ml/min in the older group. Furthermore, we also found that the measurement of the remaining kidney in the younger group was larger than that of the matched control group, but no difference was found between the measurement of the remaining kidney in the older group and that of the matched control group. Therefore, we suggest that the remaining kidney in patients over 50 years of age may be described as "a low compensatory kidney". The results from an experimental study of Wistar rats indicated that the dry and wet weights of the remaining kidneys of the younger rats which had undergone neptrectomy were 1.53×10^{-3} g/gwt, and 5.89×10^{-3} g/gwt respectively, vs 1.05×10 g/gwt and 3.82×10^{-3} g/gwt in older ones. In tissue culture using serum of younger nephrectomized rats, the amount of ^{3}H-TdR incorporation into the monolayer cells of renal cortex was 1,405.4 CPM, whereas it was 1,025.4 CPM by using serum of older nephrectomized rats. In addition, the cells of renal cortices from both younger and older rats had a similar response to the same serum from nephrectomized rats. We conclude that the compensatory growth of the remaining kidney in older rats is poorer than that of younger rats, and this difference might be related to the amount of renotropin.

Key words: Compensatory renal growth – Age factor – Renotropin

Introduction

Renal preservation is an important priciple in urology. Urologists try to avoid nephrectomy whenever possible; but the general belief is that if the other kidney is normal, the patient would have nearly the same life expectancy as those with both kidneys. However, we have observed that post-nephrectomy compensatory renal growth varies considerabilly in different patients. We also observed renal failure which developed many years after nephrectomy, which suggested that compensatory renal growth might be a variable phenomenon. Our initial impression was that the age of the patient at nephrectomy is an important factor. The present report is of our investigations comparing animal experimentation with retrospective clinical studies.

Methods and results

Animal experiments

Wistar rats were used for the experiments. The life span of these rats averages two to three years. Those of 2–3 months old were taken as the young age group and those of 12 months old as the old age group.

A comparison of the weight of the first nephrectomized kidney and that of the remaining kidney removed two weeks latter, in both young and old age groups was made. As in mammals the left kidney which is slightly heavier than the right, was removed first. The wet weight of the kidneys was immediately determined after removal and then the dry weight was determined, after 30 min in an infrared oven. To eliminate any growth factor of the animal during the interval of the two weeks between the two nephrectomies, the weight of the kidneys are expressed in renal tissue weight per gram of body weight.

The average body weight of 10 rats in old age group was 527.6 gm and that of 20 rats in young group was 125.1 gm. The comparison of the preoperative (the first removed kidney) and the postoperative (the second kidney) weight of the kidneys, in both age groups and in wet and dry weight, is shown in Table 1. In old age group, the wet and dry weight of the postoperative kidney, in comparison with the first kidney, increased by 14% and 30%. The respective increase for the

Table 1. The wet and dry weight of the resected kidneys and of the remained kidneys 2 weeks after nephrectomy

Group (*N*)	body weight (gm)		wet weight ($\times 10^{-3}$ mg/gm · BW)			dry weight ($\times 10^{-3}$ gm/gm · BW)		
	Pre	Post	K-1	K-2	Inc (%)	K-1	K-2	Inc (%)
old (10)	527.6	524.2	3.36	3.82*	14*	0.81	1.05*	30*
young (20)	125.1	170.4	4.24	5.89*	39*	1.02	1.53*	50*

Pre = preoperative; Post = 2 weeks after nephrectomy; K-1 = resected kidney; K-2 = remained kidney; Inc (%) = increased percentage; BW = body weight
* $P < 0.01$

Table 2. The scintillation count of renotropic activity in three kinds of sera

Group (*N*)	Scintillation count per min (cpm)					
Old group:						
pre. (6)	821.0 ± 116.8	(1,214.6,	814.3,	600.0,	1,065.2	440.7, 791.4)
post. (6)	1,025.4 ± 93.4	(1,213.7,	1,133.9,	762.0,	1,155.1,	706.0, 1,163.8)
sham. (6)	795.0 ± 102.1	(1,028.1,	957.1,	507.0,	914.6,	445.7, 917.2)
young group:						
pre. (6)	987.1 ± 42.4	(1,170.7,	919.1,	878.9,	973.4,	946.9, 1,033.6)
post. (6)	1,045.4 ± 75.9	(1,310.2,	1,396.4,	1,605.6,	1,289.9,	1,653.1, 1,186.2)
sham. (6)	932.6 ± 43.4	(964.2,	757.1,	1,016.9,	1,034.0,	855.0, 968.1)

Note: the number in brackets is the measured value of each rat
pre. = preoperative; post = post-nephrectomy; sham. = post-sham operation

young age group was 39% and 50%. All the differences, in both age groups and the larger increase in the young than the old age group, were significant ($P < 0.01$).

A comparison of renotropic activity of rat sera in both age groups was made. This involved the preparation of rat sera, primary cell culture of rat kidney, and scintillation count of tritiated thymidine (^{3}H-TdR) incorporation into the cultured renal cells.

Preparation of preoperative serum pool 14 rats in old age group, average body weight 517.6 mg, and 46 rats in young age group, average body weight 146.2 mg, were used. Under light ether anesthesia, blood was drawn from postorbital venous plexus through the inner canthus. Usually 5 ml of blood could be obtained from an old group rat and 2 ml from a young rat. All blood samples were set overnight under sterile condition. Blood clots were freed from the wall of the test tubes and then the samples were centrifuged for 10 min at 4,000 rpm. 0.4–0.5 ml of serum could be obtained from each ml of whole blood. The sera of the same age group were pooled and then filtered through Millipore with pore size at 0.45 μm to prevent possible contamination. The pooled sera were kept at −20°C for latter use. The rats of each age group were divided at random into two subgroups after collecting blood, and were subsequently subjected to either nephrectomy or sham operation.

Primary cell culture of cortical cell of rat kidney [1, 2]. The renal cortex taken from a 5-day old rat was minced (< 1 mm^3), into pieces and washed with Hanks Fluid, and dispersed by 0.25% trypsin and 0.02% EDTA. The digestive fluid was adjusted to pH 7.2. After several washings and centrifugation, the cells were cultured in Eagle's medium supplemented with 20% new born calf serum at 37°C. The cell growth state was observed 24 h later under an inversion microscope. The Eagle's medium was changed every 48 h. A confluent monolayer cells could be obtained of the 5th day. The cells were then incubated with serum-free medium for 24 h. These starved cells were sychronized at G1 phase. With trypsin and EDTA, the confluent monolayer cells were dispersed to a single cell suspension. After several washing and centrifugation, the cells were again suspended with Eagle's medium and the cell density was determined. They were then equivalently put into small culture bottles for latter use.

Determination of the renotropic activity. To the above culture bottles, same amount of experimental sera (preoperative, postnephrectomy and post-sham-operative) and ^{3}H-TdR were added. After incubating for 16–18 h, most cells entered S phase. The amount of ^{3}H-TdR incorporated into the DNA of the cells corresponds to the accivity of the cell growth, which in turn serves as an indicator of the renotropic activity of the testing serum. The amount of incorporated ^{3}H-TdR was determined by a Beckman Scintillation Counter following routine preparative procedures. The renotropic activity of preoperative, post-nephrectomy and post-sham operative sera as reflected by scintillation counts is shown in Table 2. For the older age group, the count was 821.0 ± 116.8 cpm for the preoperative serum, which can be considered as the control value. Value for post-nephrectomy serum was 1,025.4 ± 93.4 cpm and for post-sham-operation 795.0 ± 102.1 cpm. For young age group, the respective values were 987.1 ± 42.4, 1,405.4 ± 75.9 and 932.6 ± 43.4 cpm. These values indicate that renotropic activity of the post-nephrectomy serum is significantly higher than the control value, being 24.9% higher ($P < 0.05$) in old age group and 42.4% higher ($P < 0.05$) in young age group. The renotropic activity of the young age group is 37.3% ($P < 0.01$) higher than that of the old age group. The values for post-sham-operation sera did not show any significant difference from that of their respective controls ($P > 0.05$) (Fig. 1).

Table 3. The reactivity of different cortical cells to the same sera ($\bar{x} \pm SE$)

Group (*N*)	Scintillation count per min (cpm)							
Old group:								
pre. (7)	99.9 ± 6.7	(82.5,	98.3,	114.3,	113.3,	70.0,	117.3,	105.7)
post. (7)	188.1 ± 7.3	(172.5,	161.3,	199.3,	210.0,	172.3,	192.3,	208.7)
Inc. (%)	92.0 ± 11.1	(109.1,	64.1,	74.1,	88.7,	146.1,	63.9,	97.4)
Young group:								
pre. (7)	155.5 ± 4.5	(136.5,	141.3,	163.0,	168.0	161.7,	160.0,	158.3)
post. (7)	245.7 ± 11.1	(231.5,	202.3,	245.0,	292.7,	263.3,	259.0,	227.0)
Inc. (%)	57.8 ± 4.7	(69.2,	43.0,	50.0,	74.1,	62.8,	61.9,	43.4)

Note: the number in brackets is the measured value of each rat
pre.= preoperative sera; post. = postnephrectomy sera; Inc. = increase percentage

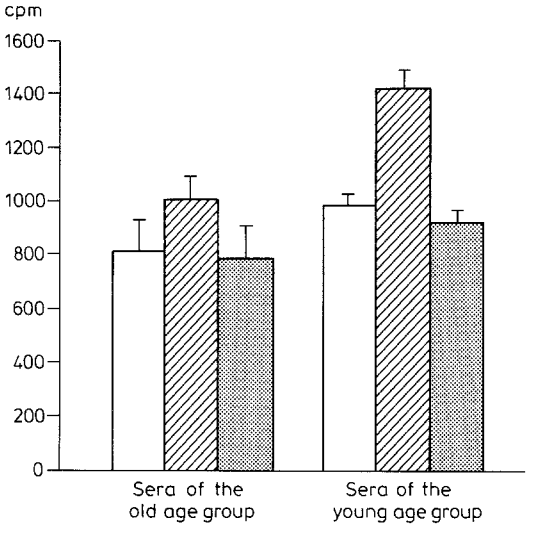

Fig. 1. The scintillation count of renotropic activity in three kinds of sera: □ pre-operation sera; ▨ post-nephrectomy sera; ⊠ post-sham-operation sera

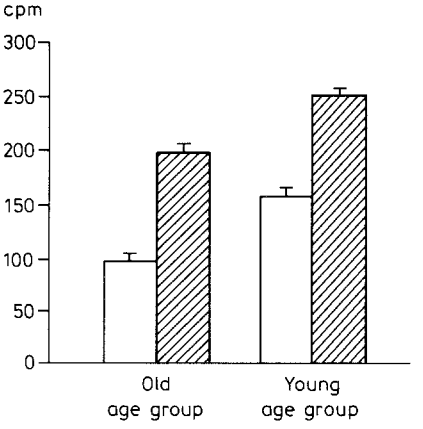

Fig. 2. The reactivity of different cortical cells to the same sera: □ pre-operation sera; ▨ post-nephrectomy sera

Determination of the difference in reactivity to renotropin between cells from old and young rat. Cell suspensions were prepared from renal cortex of female rats of old and young age groups, using the same technique as for the 5-day old rat. The preoperative and post-nephrectomy sera of the young age group were used for the determinations (Table 3). The count for old age group cell with preoperative serum was 99.9 ± 6.7 cpm and with post-nephrectomy serum 188.1 ± 7.3 cpm, an increase of 92% ($P<0.01$). For the younger age group cells the respective counts were 155.5 ± 4.5 cpm and 245.7 ± 11.1 cpm, an increase of 57.8% ($P<0.01$). It is to be noted that there is a significant difference between the two age groups even with preoperative serum, ^{3}H-TdR incorporation was higher with renal cells from young over the old by 55.7% ($P<0.01$) (Fig. 2).

Clinical studies

A retrospective study was carried out in 35 patients who had a nephrectomy at least one year prior to the study. All the 35 patients were male and the interval from nephrectomy to the present study ranged from 1–45 years, with an average of 10 years. The age of these patients at the time of the present study was 27–77 years, average 55 years. Nephrectomy had been undertaken for renal carcinoma in 22 patients, renal stone in 5, renal tuberculosis in 5, renal trauma in 2 and renal hypertension in 1 (a history of hypertension for 2 months. Nephrectomy was done at the age of 31 and the last follow-up at age 35). For all these 35 cases, clinical studies prior to nephrectomy revealed a normal contralateral kidney. For preliminary comparison, those who had nephrectomy below the age of 50 were taken as the younger group which included 19 patients and those at or above 50 years of age, 16 patients, as the older group.

Aside from general physical examination and routine urinalysis, the following examinations were carried out: (1) Blood urea nitrogen, (2) Blood creatinine, (3) Urine concentration test, (4) 12-hour endogenous creatinine clearance test and (5) Maximal length. Width at renal hilum and the thickness of the kidney were determined by B-ultrasonic scanning (Similar measurements were also carried out in 35 normal males for comparison).

Physical examination and urinalysis did not show any abnormality in both age groups. Table 4 shows the results of creatinine clearance test, blood urea and creatinine values and urine specific

Table 4. Renal function evaluations

Group (*N*)	Ccr (ml/min)	BUN (mg%)	Creatinine (mg%)	Specific gravity
50 < years old (19)	99.2 ± 8.4	17.5 ± 1.1	0.9 ± 0.06	1.018 ± 0.001
50 ⩾ years old (10)	54.9 ± 7.2	19.0 ± 1.3	1.2 + 0.13	1.015 + 0.001
P	<0.01	>0.05	>0.05	>0.05

Table 5. Comparison of ultrasonic measurement result of kidneys

Group (*N*)	Remained kidney (cm)			Control kidney (cm)		
	length	width	thickness	length	width	thickness
50 < years old (19)	11.4 ± 0.3[a]	7.3 ± 0.2[a]	5.9 ± 0.2[a]	10.3 ± 0.1	6.4 ± 0.1	4.9 ± 0.1
50 ⩾ years old (16)	10.6 ± 0.2	6.5 ± 0.1	5.0 ± 0.2	10.2 ± 0.2	6.1 ± 0.1	5.0 ± 0.2
P	<0.05	<0.01	<0.01	>0.05	>0.05	>0.05

[a] Comparing with normal control subjects, $P<0.01$

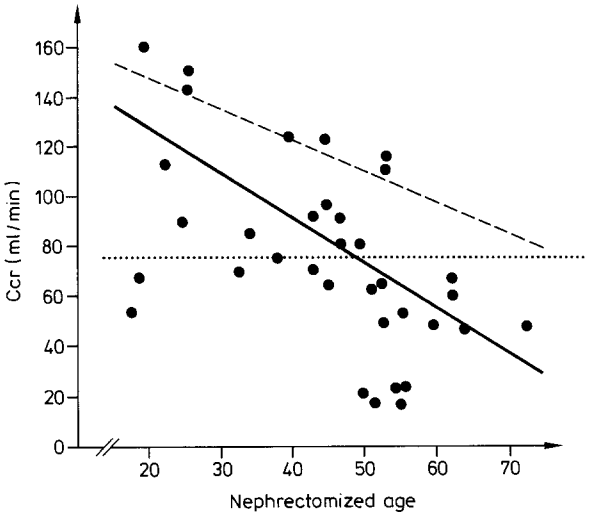

Fig. 3. The relation between Ccr and nephrectomized age: --- normal control group; ···· normal lower limit; —— after nephrectomy. $N = 35$; $r = -0.5279$ ($P < 0.01$); $Y = 144.3-1.45X$

gravfity in the two age-groups. The only difference was found in the creatinine clearance values, with a much lower value of 54.9 ± 7.2 ml/min, in the older group, as compared to 99.2 ± 8.4 ml/min in the younger age group (Fig. 3).

Comparison of ultrasonic measurement of the remaining kidney in both age group with the respective measurements in normal males of similar age group is shown in Table 5. For the young age group there is a significant increase of all dimensions of the kidney as compared to the values of those of normals of the same age group. While in the older group, there is no significant difference between those had received nephrectomy and normals. The values clearly indicated that those who had received nephrectomy before the age of 50 had a much larger remaining kidney than those received nephrectomy after 50 years of age.

Discussion

In Wistar rats, there was conclusive evidence of compensatory growth of the remaining kidney two weeks after nephrectomy, and this compensation is much less marked in older rats. There was an increase of 39% in wet weight of the kidney and 50% in dry weight in rats at 2–3 months old: while the corresponding increases were 14% and 30% in rats at 12 months old.

It is fairly well established that for compensatory growth of the remaining kidney, renotropin plays an important role [3–5]. An invitro study of the renotropic acitivity of rat sera obtained after nephrectomy, on the growth of rat cell culture was thus arranged. This study enables us to compare the renotropic activity of sera from young and old age-group rats, and also the response of renal cells from these group to the same serum.

Our study showed that the renotropic activity of post-nephrectomy serum was 42.4% higher than the pre-nephrectomy serum in the young age-group, and 29.4% higher in the old age-group. This implies that the post-nephrectomy renotropic activity was 37.3% higher in the young age-group than the old.

Response of renal cells from young and old age group using the same pre- and post-nephrectomy sera, obtained from the rats of 2–3 months old, was compared. In both age-groups there was a good response, an increase of reactivity to the post-nephrectomy serum in comparison with the pre-nephrectomy serum, by 57.8% for young rats and 92% for old rats. Even with pre-nephrectomy serum ^{3}H-TdR incorporation was

55.7% higher for cells from the young rats than the old. With post-nephrectomy serum the incorporation was also 30.6% higher for the renal cells from young rats. This probably implies that the renal cells from old are less active in terms of growth but are still responsive to renotropin. This might indicate that less renotropin activity was the primary cause of less compensatory renal growth after nephrectomy in the older animals.

All the experimental results, both in-vivo and in-vitro, point to the importance of the age factor in post-nephrectomy compensatory renal growth.

Our retrospective clinical study was in concordance with the findings obtained in animal experiments. To compare the size of the remaining kidney and the endogenous creatinine clearance test, we found that those who had nephrectomy at a younger age would have a much better compensatory growth of the remaining kidney and maintain a good renal function many years later [6–8]. In our study, the patients were divided into two groups: those below 50 and those at or above 50 years of age. This was arbitrary. The purpose was only to have the number of patients in the two groups comparable and thus to get some preliminary idea of the influence of age. It turned out that when nephrectomy was done before the age of 50, there was significant increase of all dimensions of the remaining kidney as determined by B-ultrasonic scanning. In this age-group, creatinine clearance was normal. When the nephrectomy was done after the age of 50, there was no significant compensatory renal growth of the remaining kidney and the creatinine clearance value was at much lower level, average 54.9 ± 7.2 ml/min. In fact Ogden et al. [9] based on observations on 28 kidney donors, found a similar negative correlation between renal function and the age of nephrectomy. But unfortunately the clinical significance of this finding did not receive more attention.

This study indicated that the remaining kidney in an old patient following nephrectomy should be regarded as a poorly compensated kidney. The general belief that the patient who has a clinically normal kidney on the opposite side will enjoy the same health and life expectency as a normal individual after nephrectomy is only valid if the nephrectomy is done in youth, and not always true if the nephrectomy is done later in life.

There are still other problems to be considered. Would there be any adverse effect on renotropin with chemotherapy and radiotherapy even if the nephrectomy is carried out at a young age? Would it be possible to promote or supplement the renotropic effect in an old patient who will receive nephrectomy? Some of the problems have been studied in our institute, but the study on compensatory renal growth still has a long way to go.

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