Urol Res (1998) 26:117–121
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ORIGINAL PAPER

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# CYP1A1 activity in renal cell carcinoma and in adjacent normal renal tissue

Received: 25 March 1997 / Accepted: 19 September 1997

**Abstract** Cytochrome P450-isoenzyme, CYP1A1, is responsible for the metabolic activation of several precarcinogenic environmental chemicals to their carcinogenic intermediates. Microsomal CYP1A1 activity in renal cell carcinoma (RCC) and in normal renal tissue was determined by measuring spectrofluorometrically the hydroxylation rate of benzo[*a*]pyrene. The study included 50 patients who underwent nephrectomy for RCC. Tissue specimens were taken from renal tumours and, as a control, from macroscopic normal renal tissue adjacent to the tumours. Normal renal tissues that were adjacent to poorly differentiated grade 3 tumours and/or to metastatic RCC contained significantly higher CYP1A1 activities than renal tissues next to well-differentiated ($P = 0.02$) and/or organ-confined tumours ($P = 0.001$). In conclusion, those patients who had tumours that could be considered aggressive on the grounds of poor cell differentiation or a metastatic feature of tumour, had remarkably higher CYP1A1 activities in their kidneys than the patients with less aggressive renal tumours.

**Key words** CYP1A1 · Kidney · Renal cell carcinoma

## Introduction

Renal cell carcinoma (RCC) accounts for 90% of malignant tumours of the kidney in adults. Its incidence varies world-wide, being 7.5 cases per 100 000 population in North America and Scandinavia. The aetiology of malignant renal tumours is poorly known [2]. A variety of chemical and biological agents, including lead [13] and prolonged oestrogen administration [3], have produced renal tumours in animals. No conclusive evidence has been found in humans [1, 18, 21]. RCC occurs in men twice as commonly as in women. A moderate association between tobacco use and the incidence of RCC has been reported [2].

The cytochrome P450 (CYP) superfamily plays a key role in the metabolism of many endogenous substrates as well as a wide variety of xenobiotics [14]. The CYP1A-isoenzymes, CYP1A1 and CYP1A2, are primarily responsible for the metabolic activation of a number of environmental chemicals, including polycyclic aromatic hydrocarbons (PAHs; ubiquitous in cigarette smoke, city smog and charcoal-cooked foods), to carcinogenic intermediates. Both isoenzymes exhibit a wide inter-individual variation and are clearly inducible by cigarette smoking. Of the two, only CYP1A1 is found to be expressed in extrahepatic tissues [19]. A relationship between CYP1A1 activity or its inducibility and susceptibility to chemical carcinogenesis is suspected in animal models and also in human populations [4], including numerous studies concerning malignant tumours in the lung [7, 10]. [In these previous publications CYP1A1 has frequently been called aryl hydrocarbon hydroxylase (AHH), after the commonly used laboratory method for measuring the enzyme's activity: see Materials and methods.]

In our study we wanted to clarify the expression of CYP1A1 in the kidney tissues of patients with RCC.

## Materials and methods

Kidney tissue specimens were obtained from a total of 50 consecutive patients undergoing radical nephrectomy for renal tumour. Thirty patients were men and 20 were women, the median age being 66.5 years (range 41–87 years). Histologically all the tumours were RCCs. After removal of the kidney, tissue specimens were taken

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from the tumour and from another part of the kidney which macroscopically was considered as normal. The tissue specimens were placed in small plastic bags, immediately frozen in liquid nitrogen and stored at −70°C until used for the preparation of microsomes.

The staging and grading of tumours were according to the TNM classification of the UICC [5, 22, 23]. The staging was based on the size of the primary lesion (T), its spreading to regional lymph nodes (N) and the presence or absence of distal blood-borne metastases (M). The grading was based on the degree of differentiation of cancer cells and the number of mitoses within the tumour as presumed correlates of the cancer's aggressiveness. The tumours were classified as grade 1–4 with increasing anaplasia.

Information about existing chronic diseases, current medication and smoking habits of the patients was collected.

The use of human tissues in this study was approved by the Ethics Committee of Tampere University Hospital.

### Preparation of microsomes

CYP1A1 is located in the microsomes of the cell. The microsomal fraction was prepared by differential centrifugation completed by gel filtration as previously described [8]. The microsomal protein yield per gram of renal and tumour tissue was 2.8 mg (SD 1.2) and 2.3 mg (SD 1.2), respectively. The ultimate microsomal fraction was frozen and stored at −70°C until the assay of CYP1A1 activity.

### Enzyme and protein assay

CYP1A1 activity was determined spectrofluorometrically by measuring NADPH-dependent benzo[a]pyrene (BP) hydroxylation by the method of Nebert and Gelboin [11]. The reaction mixture (1 ml) contained microsomal protein (0.2–2 mg) in 0.1 M potassium phosphate buffer (pH 7.4), magnesium chloride, NADPH, and BP in methanol as substrate. The incubation was carried out at 37°C for 30 min in the dark. The enzyme activity is expressed as femtomoles of product (equivalent to 3-OH-BP) formed per milligram of protein per minute.

The protein contents of the microsomal fractions were determined by the method of Lowry et al. as modified by Peterson [16]. The average protein contents of renal and tumour microsomes were 0.65 mg/ml (SD 0.40) and 0.55 mg/ml (SD 0.39), respectively.

### Statistics

The statistical calculations were carried out using Statgraphics software (Graphic Software Systems, STSC, Rockville, Md.). The data were summarized using the median with a range. Logarithmically transformed enzyme activities were normally distributed around the mean and the differences between the means of log CYP1A1 were tested by the two-tailed Student's *t*-test; a $P$ value $< 0.05$ was considered statistically significant.

## Results

A wide distribution of CYP1A1 activity was seen in the kidney. The specific enzyme activity in normal renal tissue varied from zero to marked activity levels up to 403 fmol/min per mg of protein, the median activity being 17.0. Among tumours the distribution of CYP1A1 was even wider, the range being from 0 to 1600; the median was 23.0. Among the studied material collected from 50 patients, in 37 samples (74%) of normal renal tissue the measured CYP1A1 activities were low (below 50). Intermediate activity, from 50 to 200, was found in 10 cases (20%), and activity considered high; (over 200) in three cases (6%). In RCCs the equivalent distribution of CYP1A1 activity was 35(70%), 10(20%) and 5(10%), respectively (Fig. 1). Men ($n = 30$) had clearly higher median enzyme activities (with a range) in both RCC [42.0 (0–1600)] and renal tissue adjacent to tumour [22.0 (0–403)] than women ($n = 20$); [15.5 (5–257) and 11.5 (0–260), respectively]. The difference between the genders as regards the tumour tissue was also statistically significant, the $P$-value being 0.02 (Fig. 2).

In our material there were eight cigarette smokers, seven of whom were men. Thus the CYP1A1 values were compared between the groups of smoking and non-smoking men. The median CYP1A1 activity in RCCs of smokers ($n = 7$) was 49.0 (2–180) and in adjacent renal tissue 13.0 (0–94). The enzyme activities in the group of non-smoking men ($n = 23$) were 28.0 (0–1600) and 27.0

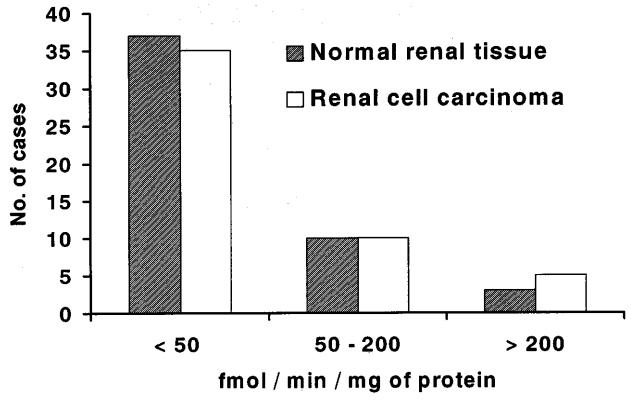


**Fig. 1** The frequency distribution of patients ($n = 50$) in the context of median CYP1A1 activities (fmol/min per mg protein) in their normal renal tissue and renal cell carcinomas

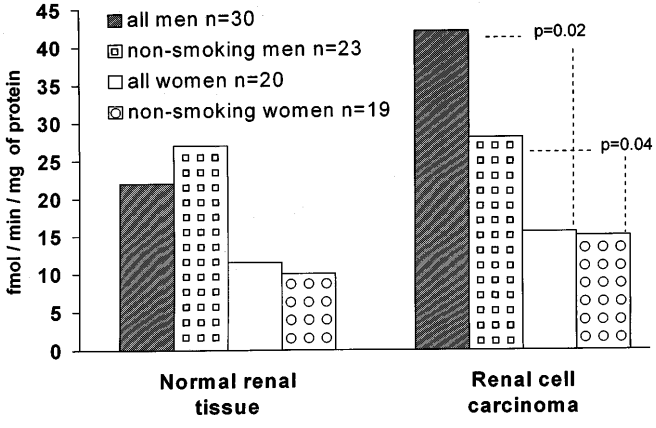


**Fig. 2** The median CYP1A1 activities (fmol/min per mg protein) in normal renal tissue and in renal cell carcinoma of men and women. The enzyme activities of the non-smoking groups are displayed separately. [The differences between the means were tested using logarithmically transformed enzyme activities (logCYP1A1), which were normally distributed around the mean. The two-tailed Student's *t*-test was used; a $P$ value $< 0.05$ was considered statistically significant]

(0–403), respectively. There was no statistical difference between the groups (Fig. 3). Cigarette smoking did not explain the higher enzyme activities measured among men, because the difference between the genders was seen also among non-smoking men and women (Fig. 2).

Among the studied cases there were 33 tumours confined to the kidney, four tumours with nodal involvement and 13 tumours with distal metastases. Patients with organ-confined tumours ($n = 33$) and patients with metastatic tumours ($n = 17$) were compared. Between these two groups there were no differences in median CYP1A1 activities of the tumours: 23.0 in organ-confined tumours and 27.5 in metastatic tumours. Nevertheless in the normal renal tissue of the patients who had metastatic tumour the median CYPA1 activity was obviously higher [50.0 (4–403)] than in the renal tissue of the patients with organ-confined tumour [13.0 (0–260); $P = 0.001$] (Fig. 4). According to the degree of differentiation of tumour cells, the tumours were divided into well-differentiated tumours of nuclear grade 1 ($n = 13$) and 2 ($n = 24$), and poorly differentiated tumours of grade 3 ($n = 10$) and 4 ($n = 3$). The measured CYP1A1 activities in those tumours and in normal kidney tissue adjacent to the tumours are presented in Fig. 5. Clearly the highest enzyme activities (median 105.0) were found in the normal kidney tissues of the patients who had poorly differentiated RCC of nuclear grade 3. Comparing the enzyme values measured in kidney tissues adjacent to grade 1, 2 and 4 tumours the difference was statistically significant ($P = 0.02$). Among the tumour tissues the highest median CYP1A1 concentration was again in grade 3 tumours; the difference compared with other tumours was not, however, statistically significant ($P = 0.69$).

The CYP1A1 contents in RCC and in normal renal tissue were considered in relation to existing chronic diseases of the patients (16 patients with hypertension, 3 with chronic coronary disease, 4 with diabetes mellitus, 3 with some other malignancy in addition to RCC: 24 patients were healthy except for the RCC). Hypertensive

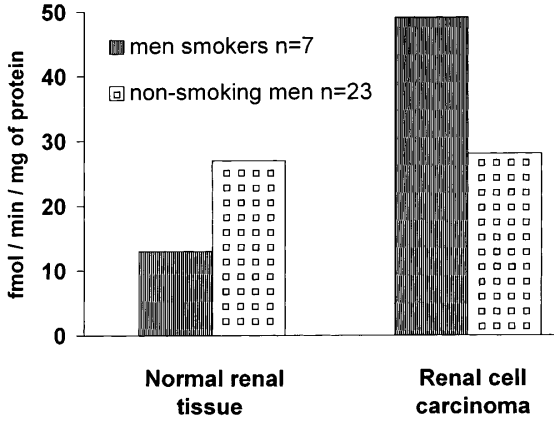


**Fig. 3** The median CYP1A1 activity (fmol/min per mg protein) in normal renal tissue and in renal cell carcinoma of smoking and non-smoking men

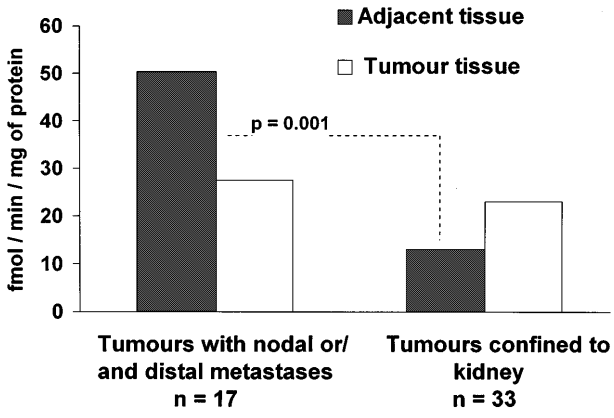


**Fig. 4** The median CYP1A1 activity (fmol/min per mg protein) in tumours with either nodal or distal metastases and in normal renal tissue adjacent to these tumours are compared with the enzyme activities in organ-confined tumours and in normal renal tissue adjacent to those. [The differences between the means were tested using logarithmically transformed enzyme activities (logCYP1A1), which were normally distributed around the mean. The two-tailed Student's *t*-test was used; a $P$ value $< 0.05$ was considered statistically significant]

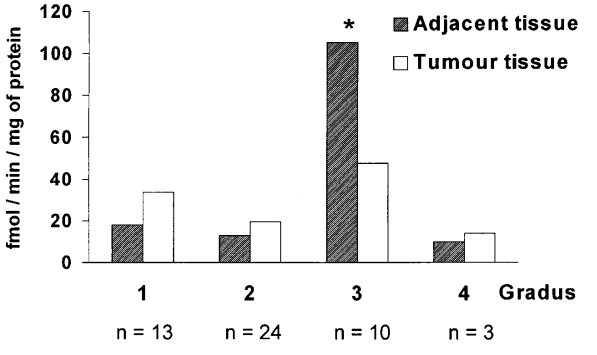


**Fig. 5** The median CYP1A1 activity (fmol/min per mg protein) in renal cell carcinomas and in normal renal tissue adjacent to the tumours with different nuclear grade. Grades 1 and 2 include tumours with well-differentiated cells and tumours with poor cell differentiation are classified as grades 3 and 4. *The measured enzyme activities in normal kidney tissue adjacent to grade 3 tumours was significantly higher ($P = 0.02$) compared with normal tissues adjacent to the other tumours. [The differences between the means were tested using logarithmically transformed enzyme activities (logCYP1A1), which were normally distributed around the mean. The two-tailed Student's *t*-test was used; a $P$ value $< 0.05$ was considered statistically significant]

patients had higher enzyme contents in their tumour tissues than others: the difference, however, was not statistically significant ($P = 0.068$) (Table 1). The medication that patients had used did not affect the measured CYP1A1 activities.

## Discussion

CYP1A1 effectively metabolizes known promutagenic and procarcinogenic environmental compounds, also present in cigarette smoke, into their ultimate carcino-

**Table 1** The median CYP1A1 activity (fmol/min per mg protein) in renal cell carcinoma (RCC) and in normal renal tissue of patients with different chronic diseases (*NIDDM* non-insulin-dependent diabetes mellitus). The differences between the groups was not statistically significant

| Patients' diseases | *n* | Tumour tissue | | Normal renal tissue | |
|---|---|---|---|---|---|
| | | Median | Range | Median | Range |
| Hypertension | 16 | 41.5 | 0–1600 | 16.5 | 0–403 |
| Coronary disease | 3 | 17.0 | 0–41 | 3.0 | 0–237 |
| NIDDM | 4 | 12.0 | 7–41 | 0.0 | 0–12 |
| Other malignancy in addition to RCC | 3 | 29.0 | 4–180 | 22.0 | 15–24 |
| No existing disease | 24 | 28.0 | 0–245 | 21.0 | 0–135 |

genic form [14]. The existence and role of CYP1A1 activity [Previously called aryl hydrocarbon hydroxylase (AHH) activity] in malignant tumours, especially in the lung, has been extensively studied [4, 7, 10]. Our present interest focused on possible differences in CYP1A1 activities in RCC and normal renal tissue.

The inducibility of CYP1A isoenzymes is genetically regulated [12], and wide inter-individual variation is therefore generally seen in CYP1A1 activities measured in human extrahepatic tissue. The wide distribution of enzyme activities in our material confirms this finding. Kellermann et al. [6] categorized individuals into three groups (low, intermediate and high) on the basis of their lymphocyte AHH inducibility. In our material, the normal renal tissue in two-thirds of cases was classified in the low activity group and the remaining one-third of cases fell more or less equally into the intermediate and high activity groups. When the material was inspected as a whole, there was no difference between CYP1A1 activity in RCC or in normal renal tissue. Certain differences, however, became obvious after division the material into subgroups.

A male predominance of RCC is well known, but as yet unexplained. A potential hormone dependence is suggested on the basis of animal experiments [3] and of the fact that low levels of steroid receptors exist in human kidney [1, 18]. Gender-related differences in the cytochrome-P450-catalysed hepatic metabolism of some steroids and drugs are substantial in the rat [24]. No observations of possible gender differences in extrahepatic CYP1A1 in humans have been published. In our study the CYP1A1 activities in the kidneys of men were higher than those in women and, in the tumour tissue, the difference between the genders was statistically significant. Although our series was small considering the wide inter-individual variation of CYP1A1, it is worth noting that the higher metabolic activity rate of procarcinogenic substances in male kidneys could be an aetiological factor explaining the greater occurrence of RCC among men.

It has previously been shown that cigarette smoke induces CYP1A1 expression and AHH activity in the human lung, lymphocytes, placenta [10, 15, 19] and myometrium [9]. The inducibility of CYP1A1 in the rat kidney following treatment with polycyclic aromatic hydrocarbon (PAH) has been verified [20]. In our study, there was an apparent increase in CYP1A1 activity in RCCs of male smokers. But no inducing effect of cigarette smoke on CYP1A1 in normal renal tissue was seen; on the contrary the measured enzyme activities in the normal kidney tissue of smokers were low. Our material contained only eight tobacco users (seven of whom were men), which is too small a number for any conclusion. In addition, levels of the individual CYP proteins are altered by exposure to a wide variety of chemicals. It was not possible to establish how many of the patients suffered exposure to passive smoking or to some other environmental inducers.

The most important single prognostic feature of RCC has been established to be the anatomical extent of the tumour at the time of surgical intervention. Patients with organ-confined tumours consistently exhibit better outcome than those with either nodal or diffuse metastatic disease [23]. Skinner et al. [22] graded RCC on the basis of nuclear morphology and found an excellent correlation between increasing loss of cell differentiation and worsened survival of patients. Recently, high AHH levels have been shown to be an independent prognostic factor in human breast cancer [17], predicting poor survival of the patient.

In our study the normal renal tissue adjacent to metastatic tumours contained significantly higher enzyme activities than the kidney tissue of patients with organ-confined tumours. In the context of tumour grading, again the normal kidney tissue near to poorly differentiated grade 3 tumour had remarkably high enzyme activity. It is thus possible that high CYP1A1 activity may have an influence on the type of carcinogenesis producing more aggressive tumours. The low CYP1A1 activities observed in tissue adjacent to undifferentiated grade 4 tumours might be due to the small numbers of specimens (only three cases). The tumours themselves, considering the extension of the tumour as well as the degree of cell differentiation, did not differ significantly one from the other as regards enzyme content. In general, the enzyme activities in tumour tissues were slightly higher than in normal renal tissue except in the normal renal tissues near to metastatic or grade 3 tumours. But it should be noted that in RCC there are commonly large areas of necrosis, which could be a potential source of error when measuring the enzyme activities of the tumours.

It is notable that the highest CYP1A1 values were measured in the normal renal tissue of patients who had tumours considered to be aggressive. The interesting question of whether those persons who have an indi-

vidual facility for a high rate of metabolic activation of xenobiotics in their kidneys would have a greater tendency to produce a cancer with a more aggressive nature, remains to be answered.

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