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Tubular PAH transport capacity in human kidney tissue and in renal cell carcinoma: correlation with various clinical and morphological parameters of the tumor

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Abstract In vitro accumulation of *p*-aminohippurate (PAH) was investigated in "intact" human renal cortical slices of normal kidney tissue and in tissue slices of renal cell carcinoma (RCC). The technique used was established in preliminary experiments on rat kidney tissue slices. In principle, the accumulation capacity is comparable in renal tissue slices of both species (slice to medium accumulation ratios between 4 and 8). In man sex differences in accumulation capacity do not exist. But, as shown in detail for rats, accumulation capacity drops with age. Tissue slices of RCC are unable to accumulate PAH actively; slice to medium ratio reaches about 1 and indicates passive PAH uptake only. Surprisingly, in tumors of stage pT1 PAH uptake is lowest, perhaps as a sign of PAH transport out of the cells. There is no difference between peripheral and central parts of RCC. Age and sex are without influence on PAH uptake in RCC tissue slices. Interestingly, the accumulation capacity of "intact" tissue of kidneys infested with RCC also depends on the severity of the tumor (stage, diameter), but not on grading and formation of metastases.

Key words Renal cell carcinoma · Renal tubular transport · Renal cortical slices · *p*-Aminohippurate · Human · Rat

Introduction

Two to three percent of all malignant tumors are renal cell carcinomas (RCCs). Until now only localized RCCs have been able to be removed successfully by radical tumor extirpation. At the time of RCC diagnosis, in 20% metastases occur distant from the primary tumor and in another 20% of RCCs, metastases can be found in the local lymph nodes [15]. About half of the patients undergoing radical tumor nephrectomy die as a consequence of their metastases [27]. It is a well-known clinical finding that RCC and its metastases are quite insensitive to any kind of cancer chemotherapy [19, 20]. This multidrug resistance is reported to be caused by expression of the *MDR1* gene and is characterized by a broad-spectrum cross-resistance to many chemotherapeutic agents [7, 21, 30]. The favorite hypothesis to explain the phenomenon of multidrug resistance is an increase in transport of chemotherapeutics out of the renal carcinoma cell [18, 23]. One method of overcoming this insensitivity is with the use of chemosensitizers, which inhibit the function of the *MDR1* gene product P-glycoprotein [6, 8, 22]. Few attractive candidates, e.g., verapamil [1, 17, 21], vitamin E [24], and tiapamil analogues [9], which should be included in clinical trials are given in the literature. Besides these therapeutic strategies there is nearly no information about the accumulation capacity of RCC, i.e., about the active uptake of a chemotherapeutic agent into the renal carcinoma cell independently of its passive diffusion. Although the latter uptake mechanism is of greater practical importance for anticancer chemotherapeutic agents [13], active uptake of these substances cannot be excluded. However, so far it has remained open as to whether or not active and/or passive uptake of anticancer chemotherapeutic agents on one hand and their active outward transport on the other hand dominates in RCC. As shown in previous experiments on renal cortical slices of rats, it is possible to stimulate the tubular accumulation capacity after pre-treatment with hormones such as dexamethasone or triiodothyronine [4] or other xenobiotics [29]. Nevertheless, these experiments were performed using *p*-aminohippurate (PAH) as the model substance; similar findings can in principle also be expected for anticancer drugs. From this point of view the question arose as to whether or not the tubular accumulation capacity in human RCC is diminished and,

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therefore, the uptake of cancer chemotherapeutics is too low for effective tumor therapy. As a first step toward clarifying this question, the accumulation capacity of normal human renal cortical tissue and that of RCC was compared using PAH as the model substance. On the basis of these experiments, further studies are planned to investigate the accumulation of chemotherapeutics in RCC and, finally, to find out whether it is possible to increase the accumulation of cancer chemotherapeutics by a suitable pre-treatment as shown for PAH in healthy rat kidney tissue.

Material and methods

Patients

Between November 1994 and December 1995, in vitro accumulation experiments were performed on human kidney slices. Both tumor tissue and "intact" renal cortical tissue of tumor-bearing kidney were obtained from 23 patients undergoing total tumor nephrectomy in the Urology Clinic, Jena. "Intact" kidney tissue means macroscopically inconspicuous material from the tumor-bearing kidney. The patients were 15 men and 8 women with an average age of 65.1 ± 7.1 years. In one patient local metastases of the lymph nodes were found, and in another patient metastases of the RCC in the brain were removed before tumor nephrectomy. Tissue samples (about 1 g of the peripheral and central part of the tumor and of "intact" kidney tissue) were stored in normal saline on ice (4°C) immediately after kidney removal. After transportation (30 min) slices were prepared as described below.

Animals

The experiments were carried out on female Wistar rats (Uje: WIST) from our institute's own outbred stock; 5- and 15-day-old animals of both sexes were used. The litters were reduced to six animals. Young animals were nursed by their dams. Adult rats were fed a standard Altromin diet and tap water ad libitum. At the time of experiment the rats were anesthetized with ether, exsanguinated and the kidneys removed and stored in normal saline on ice.

Accumulation experiments

Renal cortical slices of both humans and rats with pool sizes of about 100 mg were incubated with PAH in Cross-Taggart medium (pH 7.4, 30°C, oxygen gassing, incubation time 120 min, PAH concentration 8.5×10^{-5} M). Following incubation, PAH was determined in the supernatant fraction of the homogenate and in the incubation medium. The active uptake of PAH was expressed by the ratio between PAH concentration in the tissue and in the medium after the end of incubation ($Q_{S/M}$) in accordance with Stopp and Bräunlich [28]. Concentrations of PAH were detected using the colorimetric method introduced by Bratton and Marshall [2].

Calculations

Arithmetic means ± SEM are given. Statistically significant differences between various experimental groups were proved using Student's t-test ($P \leq 0.05$).

Results

As shown in Fig. 1, the experimental approach used in many previous experiments to characterize renal PAH accumulation on rat renal cortical slices can also be used for accumulation experiments on human renal tissue. In rats, the normal age course of the accumulation capacity is given: between days 15 and 33, after birth renal tubular accumulation of PAH becomes mature and decreases with increasing age. The "intact" kidney tissue, i.e., macroscopically normal tissue of a tumor-bearing kidney, of our youngest human patients (50 to 60 years old) accumulates PAH to the same extent as old rats. In older patients accumulation capacity also decreases. The slice to medium ratios in humans are between 2.2 and 3.8 and indicate active PAH uptake, which can be completely abolished under nitrogen gassing ($Q_{S/M} = 1$, not shown). The most important result of this study is given in Fig. 2: there is no accumulation of PAH in

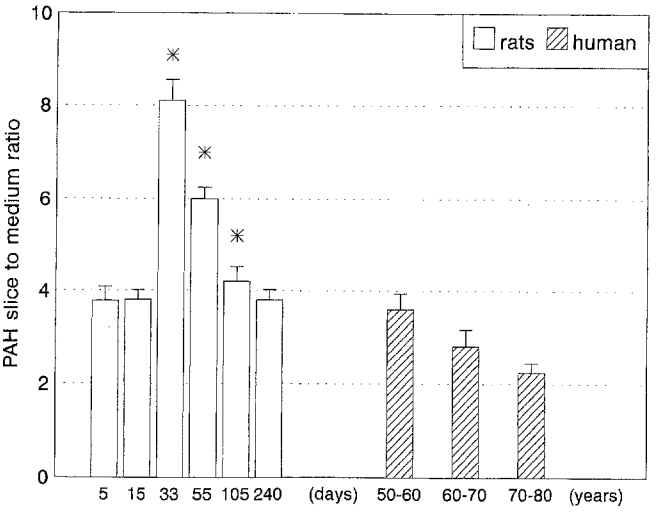

Fig. 1 Age course of PAH accumulation capacity in female rats compared to that of aged humans of both sexes. Arithmetic means ± SEM; $n = 6$ per group (rats), $n = 23$ (humans); *asterisks* indicate significant differences between consecutive age groups ($P \leq 0.05$)

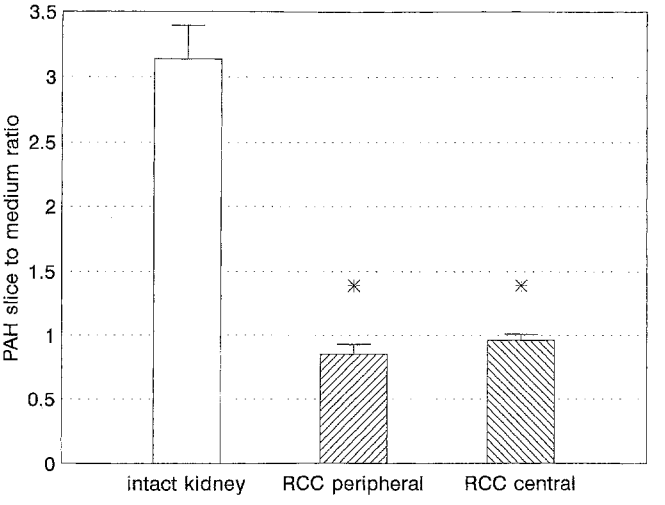

Fig. 2 PAH accumulation capacity in intact human kidney tissue and in peripheral or central parts of renal cell carcinoma (*RCC*). Arithmetic means ± SEM; $n = 23$; *asterisks* mark statistically significant reductions in RCC ($P \leq 0.05$)

tissue slices of RCC, neither in the peripheral nor in the central parts of the tumor. Correlations of the slice to medium ratios with age and sex are shown in Fig. 3. In principle there are no differences between men and women concerning the age dependence of PAH accumulation in "intact" kidney tissue. The PAH concentration in the RCC is about 1 in all age groups and both sexes, indicating a complete loss of accumulation capacity in the tumor tissue.

Surprisingly, both tumor stage and size of the tumor seem to determine the accumulation capacity of the non-tumor-bearing tissue of the same kidney (Fig. 4). The later the tumor stage, the lower is the PAH accumulation in the "intact" kidney tissue. In RCCs at stage pT1, the accumulation of PAH is obviously significantly less than 1 as a possible sign of active transport of PAH out of the cells. In this group, however, only three samples were measured.

Tumor grading and formation of metastases are not associated with PAH accumulation in either "intact" kidney tissue or RCC (Fig. 5).

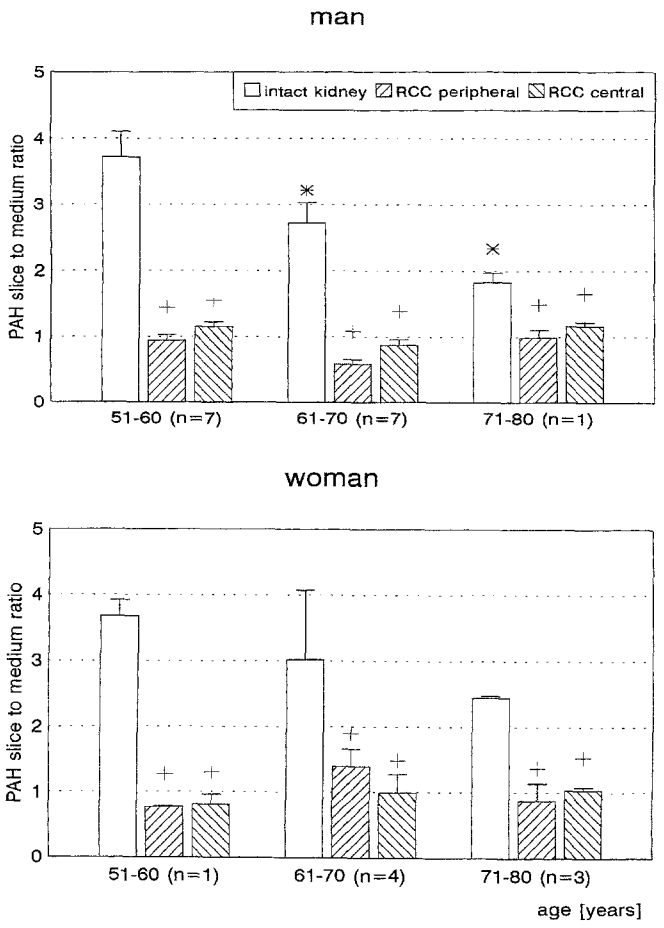

Fig. 3 Influence of sex and age on renal PAH accumulation capacity in intact kidney tissue and in peripheral and central parts of renal cell carcinoma (*RCC*). Arithmetic means ± SEM; if $n = 1$, then SEM of six measurements of the patient is given; *crosses* indicate reductions in RCC; *asterisks* mark statistically significant differences between consecutive age groups ($P \leq 0.05$)

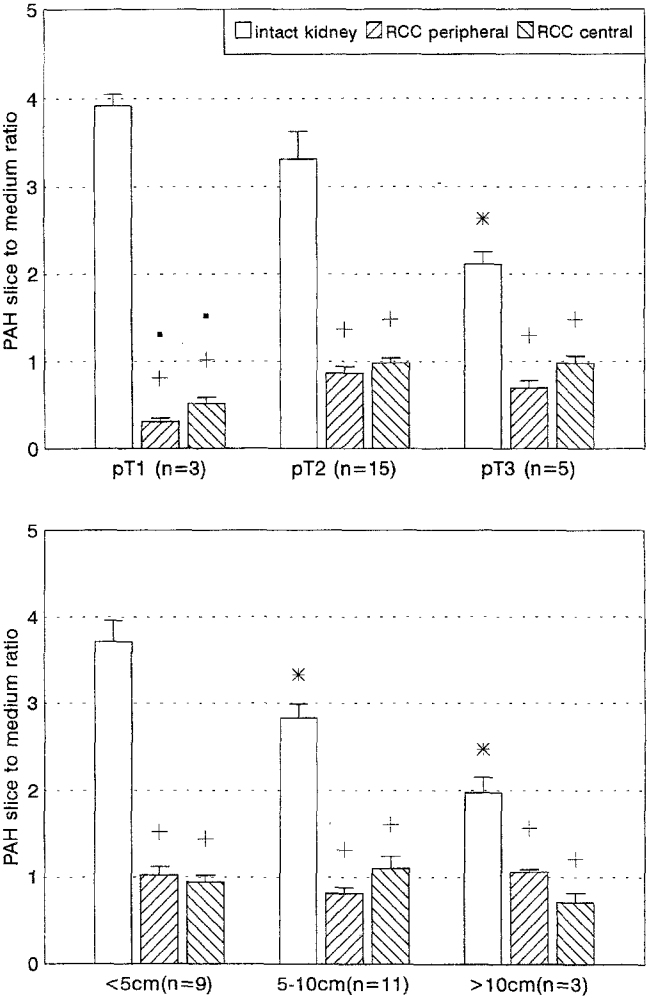

Fig. 4 Relation between tumor stage (pT1-3) or tumor size (diameter in centimeters) and renal PAH accumulation capacity in intact kidney tissue and in peripheral and central parts of renal cell carcinoma (*RCC*). Arithmetic means ± SEM; $n = 23$; *crosses* indicate significant reductions in RCC; *asterisks* mark statistically significant differences between consecutive groups of intact kidney tissue; *dots* represent significant differences between pT1 and pT2/pT3 ($P \leq 0.05$)

Discussion

The determination of the in vitro accumulation of PAH is a suitable method for the characterization of renal tubular transport capacity [28]. This study showed that it is possible to use the experimental approach for accumulation experiments established for rats for human renal tissue as well. A comparable method was reported by Fisher et al. [10] using kidney cortical slices for nephrotoxicity testing. The most marked difference between the accumulation capacity of rats and humans is the maximal extent of intracellular PAH enrichment: in rats it is about twice as high as in humans. This discrepancy could be due to four reasons:

1. Organ functions of laboratory animals are generally at a higher level than those of humans [16].

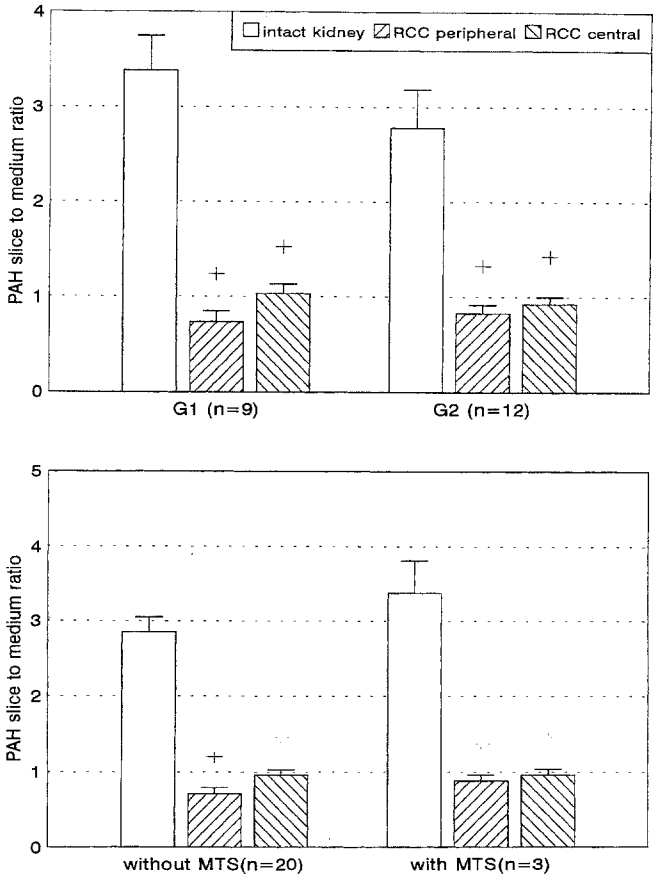

Fig. 5 Relation between tumor grading (*G1 or G2*) or formation of metastases (*MTS*) and renal PAH accumulation capacity in intact kidney tissue and in peripheral and central parts of renal cell carcinoma (*RCC*). Arithmetic means ± SEM; $n = 23$; grading was not given in two patients; *crosses* indicate significant reductions in RCC ($P \leq 0.05$)

2. It remains to be clarified whether it is justified to compare adult rats with old-aged men concerning their renal accumulation capacity.

3. It should be taken into consideration that it is possible the Cross-Taggert buffer solution is not optimal for human kidney tissue. The comparison of various buffers has not been possible as yet because of the small amounts of human RCC tissue samples.

4. Finally, kidney removal in accordance with Robsen et al. [26], the preparation of tissue samples, and the transportation of the samples from the clinic to the laboratory were somewhat time consuming. Therefore a cold ischemic period of about 30–40 min could not be avoided. Previously it has been shown for ischemic rat kidney tissue that the accumulation capacity for PAH is diminished [12].

In rats the well-known age course of PAH accumulation capacity [3] can be reproduced: it becomes mature at 1 month of life and decreases thereafter with increasing age. Also in "intact" kidney tissue of humans, accumulation capacity is inversely correlated with age, although investigations were performed only on tissue samples of old-aged patients. This finding is in good accordance with the literature [25]. Sex differences of accumulation capacity, reported for, e.g., rats [5], evidently do not exist in older humans.

Surprisingly, the PAH accumulation capacity of "intact" human kidney tissue is correlated with tumor size, especially pT status, of RCC in the same kidney, i.e., the invasive growth of the RCC obviously depresses the surrounding renal tissue before its infiltration occurs, but the definitive reason for decreased PAH transport in noncancerous tissue of tumor bearing kidney is not as yet clear.

As could be expected, the accumulation capacity of RCC tissue is completely abolished and slice to medium ratio of PAH reaches about 1. This could indicate that in RCC there is no active uptake of PAH at all, either in central or in peripheral parts of the tumor. This might reflect at least two phenomena:

1. RCC is completely undifferentiated and, therefore, unable to accumulate PAH actively. Obviously this effect occurs relatively early during the development of the RCC. As shown in Fig. 5, at later times there are no further differences regarding tumor grade and metastatic status.

2. Possibly in RCC additional transport mechanisms have been developed, transporting PAH out of the cell. Such a bidirectional transport has been shown previously for phenol red in renal tissue of healthy rats in a dose-dependent manner [11]. This out-transport might be assumed to be performed by the mechanisms responsible for the phenomenon of multidrug resistance, although the latter have been described for anticancer drugs only [14]. This hypothesis is supported by the fact that in RCC of tumor stage 1, the slice to medium ratios of PAH are below 0.5. This can be explained first of all by increased transport of PAH out of the cell, because a reduced passive influx of PAH resulting in ratios lower than 1 is less probable. Altogether there are no further differences in the accumulation capacity of RCC tissue if this parameter is correlated to tumor grading, stadium, size, and age of the patients. Thus the measured reduction of PAH accumulation capacity in RCC occurs relatively early in the course of tumor growth and remains constant at later times. However, the influence of age on the parameters mentioned cannot be decided definitively in our experiments because of the relatively low number of observations. Further experiments are necessary to investigate the anticancer drug transport into RCC as a contribution to the clarification of the reasons for multidrug resistance. It must be expected that the transport of these mostly lipophilic drugs is different from that of PAH and passive influx dominates. Nevertheless the findings of this study give an initial characterization of drug transport capacity in RCC.

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