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SHORT COMMUNICATION

RENAL METABOLISM OF HOMOLOGOUS SERUM INTERFERON

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The metabolic behaviour of homologous native and desialylated serum and urinary interferons has been investigated by using an isolated and perfused rabbit kidney, the performance of which is comparable to that in vivo. Serum native and urinary interferons disappear from the plasma perfusate with a fractional turnover rate of 1.8 and 2% and half-lives of 37 and 35 min, respectively. Serum desialylated interferon disappears much more rapidly in keeping with the finding that the glomerular sieve poses less steric hindrance and electrophysical repulsion to the passage of less anionic proteins.

These results confirm and extend our previous findings, which indicated that the kidneys have a predominant catabolic role and can explain to some extent the rapid disappearance of interferon from plasma.

interferon; renal catabolism; kidney

Several theoretical considerations discussed at length elsewhere [5,6] pointed out the possible importance of the kidneys in explaining the rapid disappearance of interferon from plasma. Indirect approaches [8,16] have supported this view and the recent use of the isolated and perfused kidney has allowed to directly demonstrate [7,9] the important role of this organ in the catabolism of human natural α and β and recombinant α_2 interferons. Since clearances of human and rabbit interferons practically overlap in the rabbit [3,11] and since human interferon can induce an antiviral state in rabbit cells [18], results obtained testing human interferon in the rabbit kidney ought to be reliable. Still it was advisable and worthwhile to investigate also the behaviour of homologous interferon. The metabolism and fate of rabbit interferon tested by perfusing the isolated rabbit kidney have been evaluated as follows.

Pooled blood interferon was collected from 25 rabbits 8 h after an intravenous inoculation of 10^9 p.f.u. of Newcastle disease virus (NDV). The serum was acidified and kept at pH 2 for 7 days in the cold, then exhaustively dialysed, concentrated against saline, centrifuged and divided into 1-ml aliquots that were kept at -80°C until used.

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Interferon was desialylated using highly purified sialidase from *Vibrio cholerae* (Behringwerke, Marburg/Lahn, F.R.G.) as previously described [4,20]. The sialic acid content was measured according to Aminoff [1]. The enzyme was inactivated by shifting the pH from 5.5 to 2.0, keeping the solution for 30 min at 37°C and then equilibrating the desialylated serum interferon by dialysis against buffered saline at a pH of 7.2.

Urinary interferon was obtained from the pooled urine of the same lot of animals. After induction with NDV there is a remarkable interferonuria [3]. The urine was acidified, dialysed and concentrated with an Amicon system model 401, using 76 mm PM-30 Amicon Diaflo membranes.

Interferon titres were obtained by a microtiter plaque reduction assay in Microtest tissue culture plates with lids (Falcon Plastic, Oxhard, California), which is a modification of that described by Campbell et al. [10]. Briefly, 2-fold dilutions in triplicates of interferon samples were dispensed on rabbit kidney cells (RL obtained by Lab-Tek) seeded at a concentration of 55 000 cells/well in Eagle's minimum essential medium (MEM) (Flow Laboratories, Irvine, Scotland) supplemented with 2% fetal calf serum (FCS). After 24 h incubation medium was removed and the cells were infected with 50 plaque-forming units (p.f.u.) of vesicular stomatitis virus (VSV, Indiana Strain) in MEM without serum. Unadsorbed virus was removed after 2 h and cultures overlayed with 1% methylcellulose in MEM with 3% FCS without phenol red. After 24–36 h plates were stained with crystal violet (1% in 70% ethanol) and plaques were counted with the naked eye. The International Reference Preparation (IRP) of rabbit serum interferon (G-019-902-528 with a defined potency of 4.0 $\log_{10}$ IU per vial) when reconstituted in 1.0 ml had a geometric mean titre of 3.75 $\log_{10}$ IU/ml (S.D. = 0.26; n = 6). In each assay an internal laboratory standard was included which had been calibrated against the IRP in 10 titrations yielding a potency of 5.39 $\log_{10}$ IU/ml (S.D. = 0.07). All titres are reported in IU/ml and are means of three or more titration results. Our rabbit serum interferon contained 5.23 $\log_{10}$ IU/ml (S.D. = 0.02) and the specific activity averaged 3.9 $\log_{10}$ IU/mg protein (S.D. = 0.22).

The rabbit serum interferon is acid-stable and is thus considered type I. Sindbis virus-induced rabbit interferon was heterogenous but the largest (68%) molecular species showed an apparent molecular weight of about 40 000 [14]. The final specific activity of urinary interferon was 4.7 $\log_{10}$ IU/mg protein and most of the activity eluted from a Sephadex G-100 column in a peak having a molecular weight of about 38 000 [3].

The perfusion apparatus and the technique have been described elsewhere [7,17]. Briefly, after cannulation of the left ureter of an anesthetized rabbit, the left renal artery was cannulated with only about 15 s of ischemia. The perfusate consisted of 125 ml homologous, fresh heparinized (7 U/ml) blood almost completely deprived of platelets and leukocytes and with hematocrit values between 25 and 28%. Insulin (5 U), creatinine (17.5 mg), penicillin G (25 000 U) and streptomycin (12.5 mg) were added immediately prior to the start of perfusion. The blood, maintained at 37.5°C, was continuously gassed with a mixture of 90% air, 5% O_2 and 5% CO_2 and the pH maintained at 7.4.

After an initial equilibration period of about 30 min, renal function remained satis-

factorily stable for 90–120 min. Owing to the very rapid clearance of interferon, it was necessary to calculate the initial concentration of interferon in the perfusate by taking into account the known plasma volume and total units of interferon added at zero time. Interferon titres sampled in the plasma at sequential time intervals were expressed as a percentage of the initial value.

Plasma and urinary creatinine was measured after precipitation with trichloroacetic acid [15], glucose by the glucose-oxidase method and hemoglobin by reading cyanmethemoglobin at 540 nm. Plasma proteins were measured according to the method of Gornall et al. [12] and urinary proteins as described by Tarantino and Marchi [19]. Plasma and urinary Na^+ and K^+ were determined by flame photometry.

Results obtained by the use of isolated organs are meaningful, if one is reasonably sure of their viability. The performance of the perfused rabbit kidney is similar to that of the kidney in vivo: mean renal arterial pressure was stable at 80–90 mm Hg, renal blood flow was about 17 ml (2.1 ml/min per g wet tissue), glomerular filtration rate (GFR) measured with creatinine was 1.9 ml/min. Fractional Na^+, K^+, glucose and water reabsorption values were similar to physiological values. Urine flow from the isolated kidney was about 4 ml/h. Proteinuria was within the physiological range and microscopic examination of urinary sediments showed rare erythrocytes.

Figure 1 (left panel) shows that the decay of homologous serum interferon from the blood was monoexponential and reproducible during the first 80 min after the initial period of equilibration. It is noteworthy that, during this period, as much as 78% of the interferon disappeared from the perfusate, so that the fractional turnover rate corresponded to a mean ± S.E. of 1.8 ± 0.2% and the half-time was 37 ± 3 min. Furthermore only 5–6 % of the interferon dose added to the perfusate was recovered in urine within 80 min. Therefore the interferon clearance/creatinine clearance (GFR) ratio was never higher than 6% implying that reabsorption of interferon by the tubular cells was almost

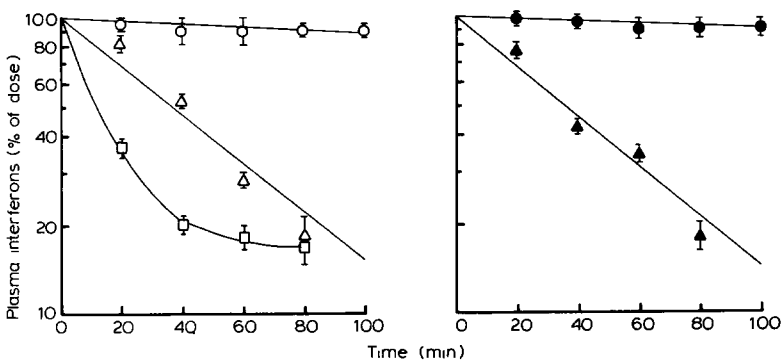

Fig. 1. Disappearance of circulating rabbit interferon in the kidney perfusion system. Left panel: system fed with serum interferon; ○, no kidney inserted in the system; △, kidney inserted; □, kidney inserted and system fed with desialylated serum interferon. Right panel: system fed with urinary interferon; ●, no kidney inserted in the system; ▲, kidney inserted. Each point represents the mean of three experiments (± SE).

complete. Both serum and urinary interferons added to blood and circulated through the perfusion system for the same length of time did not disappear and, within the variation of the assay system, the recovery was complete.

Treatment of serum interferon with sialidase released up to 86% of protein-bound sialic acid. Desialylated serum interferon disappeared (Fig. 1, left panel) with a multi-exponential curve that did not reach linearity within the time span of the experiment (90 min). When the experiment was extended beyond this time interferon titres became very low and the kidney performance deteriorated progressively. However, it is clear that the desialylated interferon contains a major fraction having a faster decay rate than the native interferon. Indeed 80% of the dose disappeared in the first 40 min of perfusion with a fractional turnover rate of about 4% and a half-life of 17 min. The subsequent very slow disappearance was probably due to the presence either of a non-desialylated interferon fraction or of a component with higher molecular weight. Titres of desialylated serum interferon remained stable when no kidney was inserted in the perfusion system.

The disappearance of urinary interferon in the perfusion system is plotted in the right panel of Fig. 1. The kinetics of disappearance were similar to those of serum interferon, i.e. monoexponential with a fractional turnover rate of 2% and a half-life of 35 min. However, with urinary interferon a higher interferon clearance/creatinine clearance (GFR) ratio (10–20%) was noted. Also interferon recovered in the urine was almost twice as high. This indicated that epithelial cells have reabsorbed less urinary interferon filtrated in the tubular fluid. It remains to be determined whether urinary interferon, almost certainly derived from plasma interferon [3], has withheld its physicochemical properties or has undergone some changes, while being in the urine, which may render it less suitable to be pinocytosed by the tubular cells.

In conclusion, our results show that the isolated and perfused rabbit kidney filtrates, reabsorbs and most likely breaks down homologous serum interferons. This confirms and extends our previous data on this subject [7–9] obtained with heterologous interferons. This is a relevant observation, since the objection had been raised to our previous work that homologous proteins could be filtrated to a different extent. Indirect approaches to this problem had already yielded results [8] that left little room for doubt.

It is interesting to note that the fractional catabolic rates measured for homologous serum and urinary interferons are 1.8 and 2%, respectively, i.e. 2–3-fold higher than the values measured for human α and β interferons [7,9]. Thus, the catabolic role of the kidney for homologous proteins is even more important than was suspected before. For a definitive assessment of the catabolic rate of human interferon the only possibility would be to employ a monkey or a human kidney.

Anyhow, the observed difference is not altogether surprising because many parameters such as molecular size, shape and electric charge, extensively discussed elsewhere [6], may influence renal filtration. A drawback of the present study is that we have tested rather uncharacterized serum and urinary interferons although we know that they are virus-

induced, acid-stable and that most of the antiviral activity has an apparent molecular weight of 38 000 to 40 000 [3,14]. From our previous studies [4,20], it is also sure that these interferons are glycosylated and contain sialic acid which can be enzymatically removed, yielding fractions with higher isoelectric points and lower electrophoretic mobility. It is known that asymmetric glycoproteins [13] can exhibit apparently higher molecular weights than the real one and this is probably so in the case of interferon. Thus, a more pronounced filtration of homologous interferon may be due to the fact that it passes more easily through the negatively charged glomerular filter than human interferon. Along the same line of ideas, as it was predicted [6] from the fact that removal of sialic acid makes interferon less anionic, desialylated interferon was filtrated more extensively than native interferon. The fact that curves were monoexponential is in keeping with the contention of Ke et al. [14] that virus-induced serum interferon is composed predominantly of a single molecular species. Experiments with artificially desialylated interferon yielded complex disappearance curves: one possible explanation for this is that the desialylation reaction was incomplete, leaving part of the interferon molecules partially sialylated.

From our previous studies [7–9,16] it is evident that interferon that has passed the glomerular filter is mostly reabsorbed by the tubular cells [2] and neither goes back in the blood stream, nor is significantly found in urine. The inescapable conclusion is that interferon is concentrated in the tubular cells and is broken down there.

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