It is my great pleasure and honour to present Prof. Fuminori Sakai’s Memorial Lecture. Since everybody may agree with the notion that Prof. Fuminori Sakai is one of ASIATOX founders and also one of pioneers in renal physiology and toxicology, this lecture consists of two parts as follows.

Remembrance of Prof. F. Sakai

Fig. 1 shows a photograph of Prof. Sakai, which was taken on May, 1995 a half year before his decease. Professor Sakai was conferred a decoration from Emperor at that time. Table 1 summarizes a short c.v. of prof. Sakai. In addition to the Table 1, several important points should be explained.

In 1981, he as Secretary General organized 8th International Congress of Pharmacology held in Tokyo together with Prof. Seturo Ebashi, President of the Congress. In 1986, Prof. Sakai organized 4th International Congress of Toxicology, ICT IV in Tokyo as President. In 1987, he became President of JSPS, and also created Japan-Korea Toxicology Symposium together with Korea Science and Engineering Foundation (KOSEF). This meeting was a prototype of Asian Society of Toxicology. He deceased at 74 years old on December 28, 1995 because of myocardial infarction.

Prof. Sakai was one of pioneers in renal physiology, since he enabled a direct access to the renal medulla in vivo (Sakai et al., 1965). Before the new method of Prof. Sakai, we could not observe the medulla in situ, because the entire kidney is covered with the cortex. In 1950s to 1960s, clarification of function of renal medulla, especially the loop of Henle and the collecting duct was a scientific fashion in renal physiology. According to this method, papillary collecting duct, Henle’s loop and vasa recta could be easily accessed for the micropuncture or microperfusion (Morgan et al., 1968; Sakai et al., 1971). Now, in every textbooks, the counter current multiplier system in the renal medulla is explained to be essential for urine concentration mechanism. Thus, Prof. Sakai’s method very much contributed to the clarification how urine can be concentrated.

Prof. Sakai’s Dr. thesis published in 1955 (Sakai) may also suggest that he was one of pioneers in nephrotoxicology. When rats were fed with yellow rice contaminated with citrinin, their renal functions were impaired. A stronger decrease was observed in para- amino hippurate (PAH) clearance, a functional indicator of renal tubular secretion than inulin clearance which means glomerular filtration rate. Prof. Sakai concluded that citrinin damaged the proximal tubule rather than the glomerulus, since PAH is excreted from the proximal tubule.

The above-stated examples are starting points of Prof. Sakai’s creation on renal physiology and toxicology. Besides them, Prof. Sakai participated a lot of sci-

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cientific discoveries in physiology, biochemistry, pharmacology and toxicology.

**Recent progress in nephrotoxicology**

**General consideration on onset and evaluation of nephrotoxicity**

The kidney is one of target organs by various toxic chemicals which include therapeutic agents, natural toxins, environmental xenobiotics etc. The reasons why the kidney is susceptible to its derangement lie in high urinary concentrations and strong tubular reabsorption of nephrotoxicants along the process of urine formation. Thus, causes of nephrotoxicity by chemical compounds are classified to roughly two groups, specific and non-specific ones.

To test nephrotoxicity by various compounds, in vivo study is one of the most reliable methods, however, understanding their toxicomechanisms is usually difficult to find. Useful parameters for in vivo test consist of urinalysis, blood chemistry and histopathology. For the identification of most suitable parameters, experimental models combining in vivo system to in vitro analyses are required.

In vitro studies can be made by using renal slices, isolated nephron segments, subcellular fractionated particles and reconstitution devices. Cells in culture are powerful materials for in vitro experiments only when their wild characters are maintained or when changes of original properties are well documented. Microperfusion of isolated well-defined nephron segments is suitable for elucidation of target site(s) and quantification of functionally dynamic changes by nephrotoxicants.

**Clarification of nephron heterogeneity**

Since the kidney is a complex organ structurally and functionally, the minimal functional unit named a nephron would be an ideal material to investigate. Morphology, physiology and biochemistry of individual segments of a single nephron were summarized for new approaches in basic nephrology in 1982 (Endou and Imai, 1982). Up to that time, main findings were obtained by physiological techniques. Since past two decades, more biochemical characteristics of nephron segments were clarified by the aids of ultramicro analytical methods.

Glomerulus: A chemical compound-induced glomerular injury was established by injecting puromycin aminonucleoside to rats resulting in nephrosis. Free radical generation could be considered as one of its mechanisms (Nosaka et al., 1996; Nosaka et al., 1997). Segment-specific findings are as follows:

Proximal tubule: There are several metabolic functions localized in this portion such as 1a, 25-vitamin D3 hydroxylation (Akiba et al., 1980), gluconeogenesis (Maleque et al., 1980; Yamada et al., 1986), 15-hydroxy prostaglandin dehydrogenation (Uchida et al., 1985), ammoniagenesis (Nonoguchi et al., 1986; Tamura and Endou, 1988), guanidinocacetate formation (Takeda et al., 1992) and P-450-dependent oxidation (Endou et al., 1982; Endou, 1983). This segment is most susceptible to nephrotoxins like cisplatin (Nosaka et al., 1992; Hosoyamada et al., 1996) and thiol compounds (Kim et al., 1997). In addition, ATP turnover in this segment is relatively high (Uchida and Endou, 1988; Jung et al., 1989), and C-kinase stimulation increases lipid peroxidation (Ha and Endou, 1992).

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**Table 1. Brief curriculum vitae of Prof. Fuminori Sakai.**

| Date and Place of Birth: April 16, 1921; Nagano, Japan |
| Education: | M.D. from University of Tokyo | 1945 |
| Ph.D. (Dr. of Med. Sci.) from University of Tokyo | 1955 |
| Academic and Professional Appointments: | | |
| 1951 | Associate Professor, Tokyo Medical and Dental University |
| 1956 | Guest Researcher, Goettingen University, Germany (2yrs) |
| 1960 | Associate Professor, University of Tokyo |
| 1963 | Research Associate, NIH, USA (1yr) |
| 1965 | Professor, University of Tokyo |
| 1982 | Director General, Japan Society of Promotion of Science (JSPS) |
| 1987 | President, JSPS |
| 1990 | Retired from JSPS |
| Deceased Date: December 28, 1995 (74 yrs old) |
Fig. 2. Hydropathy plot and proposed membrane topology of OAT1 (Sekine et al., 1997). The upper part of the figure is outside of the cell and the lower part is inside. The four symbols in the loop between 1st and 2nd transmembrane spanning domains mean possible N-glycosylation sites, and four phosphorylation sites by protein kinase C are stated in the loop between 6th and 7th domains as well.

Fig. 3. Substrate selectivity of OAT1 (Sekine et al., 1997). Inhibition of OAT1-mediated $^{14}$C-PAH uptake by various drugs and endogenous substrates were tested in the Xenopus oocyte expression system. $^{14}$C-PAH (2 μM) uptake in the presence of non-radiolabelled test substances (2 mM) are expressed as percent of control $^{14}$C-PAH uptake in the absence of other substrates (mean ± s.e.m.; n=5-8 oocytes).
Moreover, changes of intracellular free calcium concentrations are an important determinant (Jung and Endou, 1989; Jung and Endou, 1990).

Henle’s loop: In the thick ascending, loop diuretics increase prostaglandin E2 formation (Miyashita et al., 1989). These diuretics act also on short loop of the thin descending limb (Jung and Endou, 1990). This segment can produce epidermal growth factor (Oka et al., 1987) and Tamm-Forsfall glycoprotein, both of which are secreted into urine.

Distal tubule: Kallidin is produced in the connecting tubule, a second half part of the distal tubule (Tomita et al., 1981), and converted to kallikrein.

Collecting duct: The highest amounts of prostaglandin E2 are synthesized and its production decreases in spontaneously hypertensive rats (Takimoto et al., 1990). ATP (P2) receptor localized in this segment may regulate the actions of several peptide hormones like endothelin, vasopressin, bradykinin and angiotensin II (Cha et al., 1998).

**Molecular nephrotoxicology**

Recently, molecular cloning of several functional proteins has been succeeded. As shown in Fig. 2, our group has isolated a novel complementary DNA from rat kidneys that encodes a 551 amino-acid residue protein named OAT1 (organic anion transporter 1) with 12 putative membrane spanning domains (Sekine et al., 1997). When expressed in *Xenopus laevis* oocytes, OAT1 mediated sodium independent PAH uptake. The uptake rate of PAH was increased by outwardly directed dicarboxylate gradient, consistent with the idea that OAT1 is an organic anion/dicarboxylate exchanger. Fig. 3 represents remarkable wide substrate selectivity of OAT1, covering endogenous substrates such as cyclic nucleotides, a prostaglandin and uric acid and a variety of drugs with difference structures (e.g., antibiotics, a nonsteroidal anti-inflammatory drug, diuretics, an antineoplastic drug and a uricosuric drug). Furthermore, an anionic fungal nephrotoxin, ochratoxin A (Jung and Endou, 1989) also inhibits PAH uptake in oocytes expressing OAT1. Thus, several nephrotoxic organic anions could be screened by this oocyte system. The Northern blot analysis and in situ hybridization revealed that OAT1 is exclusively expressed in the middle portion of the proximal tubule in the kidney. Since simultaneous administration of two kinds of drugs (e.g. aminoglycoside and loop diuretic) induces renal failure, it would be possible to clarify drug/drug interaction using OAT1-expressed oocytes. Since we could isolate a cDNA from human kidney (submitted), stable expression of human clone in cultured cells may enable to screen many kinds of anionic compounds within a short time before their direct application to human.

These results suggest that isolation of functionally active proteins like OAT1 will facilitate elucidation of the molecular basis of drug kinetics and the development of new drugs lacking unwanted nephrotoxic effects.

**REFERENCES**


