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Effect of Uranyl Nitrate-Induced Acute Renal Failure on the Pharmacokinetics of Sulfobromophthalein in Rats

Gun-Hwa Park and Chang-Koo Shim[§]

College of Pharmacy, Seoul National University, Seoul 151-742, Korea
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Abstract□The effect of acute renal failure (ARF) on the pharmacokinetics of sulfobromophthalein (BSP) was investigated in order to elucidate if renal failure modifies the hepatic metabolism of drugs. ARF was induced by intravenous (*iv*) injection of uranyl nitrate (UN) to rats (5 mg/kg) five days before the experiment. Area under the plasma concentration-time curve (*AUC*) of BSP after portal vein (*pv*) injection increased by 2-fold and total body clearance (CL_t) decreased one half ($p<0.01$) in UN-induced ARF (UN-ARF) rats compared to the control rats. But the plasma disappearance of BSP after *iv* injection did not differ significantly between control and UN-ARF rats. Since BSP is excreted *via* the liver, CL_t representd the approximate hepatic clearance of BSP. Therefore, the decrease in CL_t represents a decrease in hepatic intrinsic clearance (CL_{int}) for BSP since plasma free fraction (f_p) of BSP was not affected by UN-ARF. The content of hepatic cytoplasmic Y-protein, which catalyzes BSP-glutathione conjugation and limits the transfer of BSP from blood to bile, increased significantly ($p<0.01$), however its binding activity (BA) for BSP was decreased significantly ($p<0.01$) by UN-ARF. The decrease in CL_{int} might have some correlation with the changed characteristics of hepatic Y-protein, specifically its decreased BA for BSP.

Keywords□Acute renal failure (ARF), uranyl nitrate, sulfobromophthalein (BSP), pharmacokinetics, protein binding, Y-protein.

The effect of acute renal failure (ARF) on the hepatic metabolism of drugs has been studied by many investigators. The content of mocrosomal P-450 enzymes decreased in renal failure, therefore, the pharmacological effect of zoxazolamine[1], ketamine[2] and hexobarbital[3] were sustained. The demethylation of aminopyrine[1-4], benzphetamine[1] and *p*-nitroanisole[3] and the hydroxylation of acetanilide[3] decreased in ARF. Plasma clearance and hepatic uptake of indocyanine green (ICG)[5-7] and sulfobromophthalein (BSP)[8] decreased by glycerol-or surgically induced ARF. Recently hepatic intrinsic clearance of propranolol was reportedly decreased in uranyl nitrate-induced ARF (UN-ARF)[9,10].

In renal failure patients, phase I metabolism (degradative biotransformation)[11-18] and phase II metabolism (synthetic biotransformation)[19-23] of some drugs decreased. On the other hand, the metabolism of biphenyl[24], diphenylhydantoin[25], thiopental[26], digoixn[27,28], phenylbutazone[29] and antipyrine[30] increased and hydrolysis of atropine[31], conjugation of chloramphenicol[31] and oxidation of meprobamate[31] were not altered in uremic patients.

BSP clearance from the plasma is an important index for the diagnostic evalution of the liver function. BSP is bound to plasma protein in the blood[32], taken up into the hepatocytes and bound to hepatic cytoplasmic Y-protein which is glutathione (GSH) S-transferase[33]. About 80% of BSP is excreted into bile as GSH conjugate[34-38]. GSH conjugation, which is catalyzed by GSH S-transferase, is the rate-limiting step in the overall transfer of BSP from blood into bile[39].

The effect of UN-ARF on the hepatic metabolic function, especially on GSH conjugation was studied in rats: pharmacokinetics, plasma protein binding and hepatic cytosolic protein binding of BSP were compared between the control and UN-ARF rats.

EXPERIMENTS

Chemicals

Sulfobromophthalein sodium salt (BSP, Sigma Chemical Co.), Sephadex G-75 (superfine grade, Phar-

§ To whom all correspondence should be address.

macia Free Chemicals) and uranyl nitrate-6-hydrate (UN, Merck Co.) were used as received.

Induction of UN-ARF

Male Wistar albino rats, weighing 230-300g, were obtained from the Laboratory Animal Center of Seoul National University. UN-ARF was induced by *iv* injection of UN solution in physiological saline at a dose of 5 mg/kg five days before the experiment[4,40,41].

Pathophysiological assay

The carotid arterial plasma was assayed for blood urea nitrogen (BUN) by the indophenol method[42,43] using a commercial kit (Urea NB kit; Wako Pure Chemical Ind. Co., Tokyo). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were assayed by the modified Reitman-Frenkel method[44] using commercial kits (Dri-Stat AST and ALT kits; Beckman Instruments Co., Fullerton, Calif.). Total protein, albumin, creatinine and alkaline phosphates were assayed by autoanalyzer (SMA 12/60; Technicon Instruments Co., Basingstoke, England).

Plasma protein binding

Plasma was seperated from the blood obtained through the carotid artery by centrifugation (6000×g, 10 min) at room temperature. The plasma free fraction (f_p) of BSP was determined by equilibrium dialysis at 4°C for 40 hr using semipermeable membrane (Type 18/32; Visking Co., Chicago, III.) against Tris-HCl buffer (pH 7.4). BSP was added to the protein chamber. The f_p was confirmed to be constant between 30 and 40 hr of dialysis at 4°C. The BSP concentration in the protein chamber after dialysis was 40 μg/m*l* and in the range of the *in vivo* concentration of BSP.

Intravenous (iv) and portal venous (pv) administration of BSP

Under light ether anesthesia, the femoral or pyloric vein and femoral artery were cannulated with polyethylene tubings (PE-50, Intramedic, Clay Adams, USA) for BSP administration and blood sampling, respectively. BSP was injected at a dose of 20 mg/kg (2 m*l*/kg) in both studies. Blood samples (0.25 m*l*) were withdrawn from the femoral artery *via* PE-50 catheter at 1, 2, 3, 4, 5, 7, 10 and 15 min after the injection. Plasma samples were obtained by centrifuging the blood samples at 6000×g for 10 min. To 0.1 m*l* of plasma samples, 3.0 m*l* of 0.05 N-NaOH was added and the absorbance at 578 nm was measured spectrophotometrically. BSP concentration in the plasma was calculated using the calibration curve prepared with blank plasma in the same manner.

Binding to cytoplasmic proteins

Under light ether anesthesia, rats of control group (n=4) and UN-ARF group (n=4) were killed by bleeding from a carotid artery. Thereafter, the liver was perfused with ice-cold 0.95 (w/v) saline through the portal vein and rapidly removed, and 25% (w/v) homogenate was prepared in 0.25 M sucrose-0.01 M phosphate buffer, pH 7.4 using a motor-driven teflon pestle glass homogenizer (SM-3 type, Omega-Electric Co., Japan). The homogenate was centrifuged at 110,000×g for 120 min at 4°C. The supernatant fraction was removed and either used immediately or stored at −40°C. A 2 m*l* portion of the supernatant was mixed with 2 μmole BSP and placed on a Sephadex G-75 superfine column (2.2×40 cm). Elution was performed with 0.01 M phosphate buffer (pH 7.4). at the flow rate of 10 m*l*/hr at 4°C. Protein concentration was estimated by absorbance at 280 nm, and protein-bound BSP by absorbance at 578 nm after alkalization with 0.1 m*l* of 5 N-NaOH. X-, Y- and Z-fractions were collected following the nomenclature of Levy *et al*[45].

Pharmacokinetic analysis

Pharmacokinetic parameters in terms of two compartment open model[46] for BSP were calculated with a non-linear iterative least sqares method using a MULTI program[47]. Area under the plasma concentration-time curve (AUC) was calculated by trapezoidal rule and the total body clearance (CL_t) was calculated by

$$CL_t = Dose/AUC \qquad \text{(Eq.1)}$$

Statistical analysis

All means are presented with their standard error (mean ± SEM). Student's *t*-test was utilized to determine a significant difference between the control and UN-ARF groups.

RESULTS AND DISCUSSION

The pathophysiological changes caused by UN-ARF are shown in Table I. Total protein and albumin concentration in the plasma decreased significantly ($p<0.05$ and $p<0.01$ respectively), but were still in the normal ranges. BUN and creatinine concentrations showed significant ($p<0.001$) increasses. On the other hand, no significant differences were observed

Table I. Pathophysiological changes by uranlyl nitrate-induced acute renal failure (UN-ARF)[a]

Parameter	Control (n=9)	UN-ARF (n=2)
Total protein, g/dl	6.06±0.33	5.25±0.18[b]
Albumin, g/dl	3.55±0.18	2.93±0.09[c]
BUN, mg/dl	23.93±2.32	221.80±17.24[d]
Creatinine, mg/dl	0.73±0.08	7.46±0.43[d]
Alkaline phosphatase, mU/dl	200.44±13.30	189.33±23.80
GPT, mU/dl	1.78±1.00	31.36±4.68
GOT, mU/dl	128.11±8.95	144.36±17.53
Body wt before treatment, g	274.38±17.05	274.33±8.31
Body wt after treatment, g	283.14±11.32	254.39±7.53[c]
Liver wt, g/kg body wt	33.03±0.92	31.32±1.04

[a]Expressed as mean±SEM. [b]$p<0.05$, [c]$p<0.01$, [d]$p<0.001$.

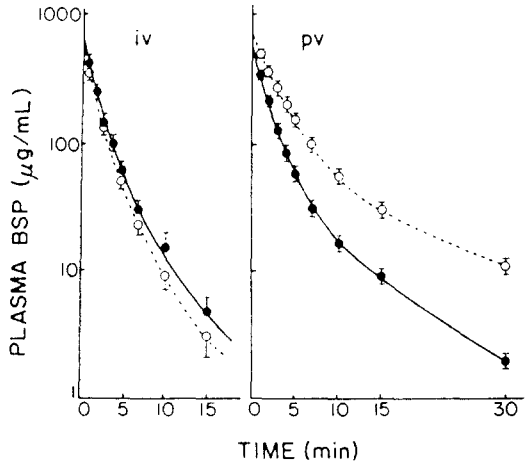

Fig. 1. Mean plasma concentration-time curves of BSP after *iv* (left) and *pv* (right) administration of 20 mg/kg dose to the control (○, n=4) and UN-ARF (●, n=3) rats. Bars represent mean±SEM.

in plasma GOT, GPT and alkali..e phophatase activities. Body weight decreased significantly ($p<0.01$), but no significant difference was observed in the liver weight. Since the changes in pathophysiology were found to be highly reproducible, UN-ARF model was used as a ARF model in this study.

Plasma disappearance curves of BSP after *iv* and *pv* administration to control (n=4) and UN-ARF (n=3) rats are shown in Fig. 1 No significant difference in the plasma concentration was observed after *iv* administration of BSP between the control and UN-ARF rats. But a typical delay of plasma disappearance of BSP was observed in the UN-ARF rats after *pv* administration.

Pharmacokinetic parameters computed by a non-linear iterative least squares method[47] for the data illustrated in Fig. 1 are listed in Table II. There were no significant changes in *iv* parameters by UN-ARF. But in *pv* administration, UN-ARF rats showed significant decreases in the first order elimination rate constant, K_{20} ($p<0.05$), and CL_t ($p<0.001$). AUC of BSP following *pv* administration (AUC_{pv}) was significantly ($p<0.01$) increased by UN-ARF. It is consistent with previous reports for propranolol[9,10] in UN-ARF.

There were no significant changes in the unbound fraction (f_p) of BSP in the plasma (Table I), distribution rate constants (K_{12}, K_{21}), distrbution volumes (V_c, V_p, Vd_{ss}), biological half-lives ($t_{1/2}\alpha$, $t_{1/2}\beta$) by UN-AFR in both administrations (Table II). It is contrary to Bowmer *et al*[8]. Who reported significant increases in $t_{1/2}\beta$, V_c and Vd_{ss} togehter with significant decrease in K_{12} and K_{20} by glycerol-induced ARF (G-ARF). It might be due to the different pathophysiology of G-ARF from UN-ARF. Indeed, there were no significant changes in pharmacokinetic parameters of *iv* propranolol by UN-ARF[9,10] which is consistent with our study. Table II also shows that some pharmacokinetic parameters (K_{21}, V_p, Vd_{ss} and $t_{1/2}\beta$) are significantly larger for *pv* BSP, the mechanism of which is not clear at present.

AUC changes by UN-ARF can also be interpreted by the well-stirred model[48]. According to this model, the AUC of BSP following *iv* administration (AUC_{iv}) can be expressed as

$$AUC_{iv}=D\cdot(Q+f_p\cdot CL_{int})/Q\cdot f_p\cdot CL_{int} \qquad \text{(Eq.2)}$$

were D is the dose, Q is the hepatic plasma flow and CL_{int} is the intrinsic clearance of the unbound BSP. The $f_p\,CL_{int}$ can be replaced by CL_t. Eq.2 shows that AUC_{iv} dose not adequately reflect the CL_{int} since it depends on D, Q, f_p and CL_{int}. But if $f_p\cdot CL_{int}>>Q$, AUC_{iv} is limited solely by Q; and if $f_p\cdot CL_{int}<<Q$, AUC_{iv} is limited by $f_p\cdot CL_{int}$.

On the other hand, AUC of BSP following *pv* administration (AUC_{pv}) can be alway given as

$$AUC_{pv}=D/f_p\cdot CL_{int} \qquad \text{(Eq.3)}$$

Thus AUC_{pv} depends only on D, f_p and CL_{int}, but is independent from Q. Therefore, AUC_{pv} adequately reflects the $f_p\cdot CL_{int}$. Consequently, increased AUC_{pv} of BSP observed in UN-ARF rats must conclusively be due to the decrease in CL_{int} since f_p in the study was not changed by UN-ARF. CL_{int} decreased from

Table II. Effect of UN-ARF on the pharmacokinetics of BSP administered intravenously (*iv*) and portal venously (*pv*) in rats[a]

Parameter	*iv*		*pv*	
	Control	UN-ARF	Control	UN-ARF
No. of animals	4	3	4	3
K_{20}, min^{-1}	0.55 ± 0.03	0.58 ± 0.03	0.49 ± 0.06	$0.25 \pm 0.02^{b,e}$
K_{12}, min^{-1}	0.11 ± 0.04	0.09 ± 0.03	0.14 ± 0.08	0.10 ± 0.00
K_{21}, min^{-1}	0.30 ± 0.03	0.30 ± 0.05	0.12 ± 0.01^{f}	0.11 ± 0.01^{f}
AUC, $\mu g \cdot min \cdot ml^{-1}$	1551.5 ± 270.8	1320.8 ± 103.7	1502.6 ± 75.52	926.9 ± 172.4^{c}
CL_t, $ml \cdot min^{-1} \cdot kg^{-1}$	13.7 ± 2.6	15.4 ± 1.2	13.4 ± 0.7	6.9 ± 0.4^{d}
V_c, $ml \cdot kg^{-1}$	24.9 ± 3.5	26.4 ± 1.2	30.1 ± 4.7	28.7 ± 4.7
V_p, $ml \cdot kg^{-1}$	7.3 ± 2.5	8.2 ± 3.0	29.8 ± 12.1^{e}	25.8 ± 3.5^{f}
Vd_{ss}, $ml \cdot kg^{-1}$	32.2 ± 1.5	33.4 ± 1.3	59.9 ± 11.1^{e}	54.5 ± 8.1^{f}
$t_{1/2}\,\alpha$, min	0.96 ± 0.07	0.95 ± 0.06	1.20 ± 0.26	1.80 ± 0.08^{g}
$t_{1/2}\,\beta$, min	3.07 ± 0.09	3.09 ± 0.24	8.06 ± 0.79^{g}	10.01 ± 0.48^{g}

[a]Doses of BSP were 20 mg/kg rat. Data are expressed as mean ± SEM. [b]$p<0.05$, [c]$p<0.001$, [d]$p<0.01$; Significantly different from pv Control. [e]$p<0.05$ [f]$p<0.01$ [g]$p<0.001$; Significantly different from those of *iv* studies.

1023.9 to 488.1 ml/min/kg when calculated according to Eq.3. BSP is almost eliminated through the liver[34-38], therefore, the decrease in CL_{int} indicates the decrease in hepatic intrinsic clearance for unbound BSP by UN-ARF.

Considering a possible first-pass biotransformation in the liver, AUC_{pv} is expected to be smaller than AUC_{iv} when equal doses of BSP are administered to control rats. On the contrary, there was no significant difference between AUC_{iv} and AUC_{pv} in control rats (Table II). It may occur in the limiting case of $f_p \cdot CL_{int} << Q$. But if this is the case, AUC_{iv} in Eq.2 can be simplified as $D/f_p \cdot CL_{int}$ and, as like AUC_{pv} in Eq.3, any changes in $f_p \cdot CL_{int}$ caused by UN-ARF will be reflected adequately in AUC_{iv}., however AUC_{iv} was not changed significantly by UN-ARF in spite of significant ($p<0.001$) decrease in CL_{int} for BSP *pv* adminstered (Table II).

Therefore, the assumption, $f_p \cdot CL_{int} << Q$, was denied. The difference in the surgical operations between *iv* and *pv* administrations seemed to be a possible explanation for the lack of difference in AUC_{iv} and AUC_{pv} in this study. Difference in some pharmacokinetic parameters as like K_{21}, V_p, Vd_{ss} and $t_{1/2}\,\beta$ between *iv* and *pv* studies might be the reflection of the different experimental conditions in both administrations. If the sham operation had been performed for *iv* study, AUC_{iv} of BSP might have overrun AUC_{pv} more clearly in control rats.

Actually, Q of control rats calculated from the data of Katayama *et al*[9] and of the liver weight (Table I) was about 23 ml/min/kg, which was comparable to $f_p \cdot CL_{int}$ or CL_t of *iv* administered BSP. In this case, a small change in CL_t may not be reflected sensitively in AUC_{iv}. It explains why AUC_{pv} reflects the change in CL_t or $f_p \cdot CL_{int}$ more sensitively than AUC_{iv}. Therefore, AUC_{pv} seems to be a better measure of CL_t or $f_p \cdot CL_{int}$ change than AUC_{iv}, although it could not be compared directly with AUC_{iv} since it was determined under different experimental conditions in this study.

In order to elucidate the mechanism of decreased CL_{int}, gel filtration chromatography was performed against 25% (w/v) liver supernatant-BSP mixture. Representative chromatograms for the control and UN-ARF rats are shown in Fig. 2. The chromatograms of the control rats were in agreement with the reported results[49,50]. Following the nomenclature of Levy *et al*[45], the first, second and third peaks were labeled X-, Y-, and Z-protein respectively. In the control rats, most of the BSP was bound to the Y-protein and less was bound to X- and Z-proteins. The peak absorbance at 280 nm of the Y-protein and the ratio of X/Y proteins were increased significantly ($p<0.05$ and $p<0.01$ respectively) by UN-ARF (Table III).

In order to examine the binding of BSP to each protein, the binding activity (BA) was calculated as folllows:

$$BA = Abs(578)/Abs(280) \qquad \text{(Eq.4)}$$

where $Abs(578)$ and $Abs(280)$ represent the peak aborbance of BSP bound to the protein at 578 nm and that of the protein at 280 nm, respectively. BA of Y-fraction decreased significantly ($p<0.05$) in UN-ARF

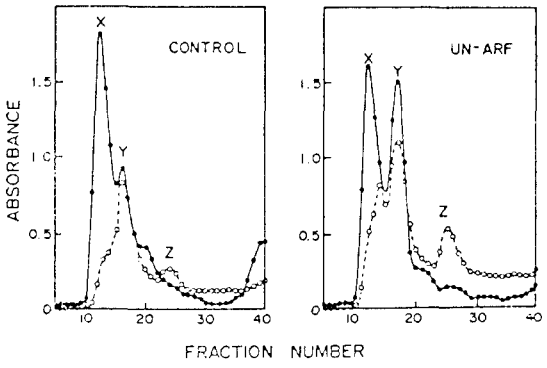

Fig. 2. Representative elution patterns of BSP and hepatic cytosolic proteins of the control (left) and UN-ARF (right) rats. A 2 μ mole of BSP was mixed with 2 ml of the supernatant of 25% (w/v) liver homogenate and eluted on Sephadex G-75 superfine column.
Key: (●) absorbance at 280 nm (proteins), (○) absorbance at 578 nm (BSP).

Table III. Effect of UN-ARF on the binding of BSP to liver cytosolic fraction[a].

Absorbance	Control	ARF
X-protein[b]	1.70±0.21	1.68±0.10
Y-protein[b]	0.98±0.17	1.63±0.14*
Y/X[c]	0.57±0.04	0.97±0.03**
BA of Y-protein[d]	0.86±0.05	0.58±0.08*

[a]Expressed as mean±SEM. Sephadex G-75 gel filtration was performed on the supernatsant of a 25% (w/v) liver homogenate after mixing with 2 μ mole of BSP. [b]Peak absorbance at 280 nm. [c]Absorbance ratio of X, Y-protein. [d]Calculated according to Eq.4. *$p<0.05$, **$p<0.01$.

rats, but those of X-and Z- fractions were not changed significantly by UN-ARF (Table III).

It was suggested that two cytoplasmic organic anion binding proteins, Y-protein (ligandin) and Z-protein, are important determinants in the transport of many organic anions, especially BSP, from the plasma into the liver[50,51]. In the present study, in spite of its significant ($p<0.05$) increase in content and apparently unchanged total binding with BSP, *BA* of Y-protein decreased significantly ($p<0.05$) in UN-ARF-rats (Table III). The increase in Y-protein might be the result of decreased protein metabolism or renal excretion induced by UN-ARF.

Plasma clearnces of ICG[5-7] and BSP[8] were reported to be decreased by glycerol- or surgically induced ARF. Decrease in hepatic uptake of ICG and BSP was suggested as a possible mechanism of the decrease[5-8].

Renal failure can induce changes in protein metabolism[51] and Wernze *et al*[52] suggested that altered hepatic protein metabolism may be responsible for the decreased hepatic uptake. Yates *et al*[7] suggested that alteration in the intercellular concentration of hepatic cytoplasmic concentration could possibly alter the influx of their ligands into the hepatocyte.

Indeed, the content of Y-protein was increased by UN-ARF. Nevertheless, total binding of Y-protein with BSP was not changed and *BA* of Y-protein was decreased significantly ($p<0.05$) by UN-ARF. Therefore, the decrease in *BA* of Y-protein, a ligandin for BSP, seems to be responsible for the decreased hepatic uptake of BSP and eventually for the decreased hepatic CL_{int} and CL_t of BSP in UN-ARF rats.

In conclusion, significant ($p<0.001$) decreases in hepatic metabolic function such as CL_{int} for BSP were found in UN-ARF rats and its possible correlation with decreased binding activity (*BA*) of Y-protein was suggested in this study.

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