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# Effects of Guanosine on the Pharmacokinetics of Acriflavine in Rats Following the Administration of a 1:1 Mixture of Acriflavine and Guanosine, a Potential Antitumor Agent

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Preclinical studies are currently underway to examine the potential antitumor effects of a 1:1 mixture of acriflavine (ACF; CAS 8063-24-9) and guanosine. Guanosine potentiates the anticancer activity of some compounds. However, the effects of guanosine on the pharmacokinetics of ACF in mammals are unknown. Therefore, this study investigated the effects of guanosine on the pharmacokinetics of ACF after administering a 1:1 mixture of ACF and guanosine in rats. The rats were given either 10 mg/kg of the mixture or 5 mg/kg ACF via an intravenous bolus injection; or 30 mg/kg of the mixture or 15 mg/kg ACF intramuscularly. An HPLC-based method, which was validated in this laboratory, was used to analyze the levels of trypaflavine (TRF) and proflavine (PRF) in the plasma, bile, urine, and tissue homogenates. It was found that TRF and PRF were rapidly cleared from the blood and transferred to the tissues after the i.v. bolus or i.m. injection of the combination mixture. Both TRF and PRF were found to be most highly concentrated in the kidneys after the i.v. bolus or i.m. injection, followed by slow excretion to the bile or urine. Guanosine had no effect on the plasma disappearance of TRF or PRF after the i.v. bolus injection. However, guanosine led to a prolongation of the plasma levels of PRF after the i.m. administration of the combination mixture, resulting in a 2 fold increase in the bioavailability (BA) of PRF. The concentrations of TRF and PRF in all the tissues examined were similar in the groups given the mixture and ACF. However, guanosine led to a prolongation of the biliary and urinary excretions of both TRF and PRF after the i.v. bolus (1.25 fold) or i.m. (1.5-2.4 folds) injection. These prolonged effects of guanosine on the plasma disappearance or urinary excretion of TRF and PRF might be one reason for the enhanced antitumor effects of ACF. However, more study will be needed to further examine this potential mechanism.

**Key words:** Acriflavine, Guanosine, Pharmacokinetics, Excretion, Distribution

## INTRODUCTION

Acriflavine (ACF; CAS 8063-24-9), which is a 2:1 mixture of 3,6-diamino-10-methylacridinium chloride (trypaflavine, TRF) and 3,6-diaminoacridinium (proflavine, PRF), has been used as a trypanocidal and antibacterial agent (Fig. 1). ACF has also been examined as a potential anti-infective agent in fish (Yu *et al.*, 1997; Plakas *et al.*, 1988) and has been shown to eradicate the Friend virus in combination with other agents (Macadam and Williamson 1974; Mathe *et al.*, 1994). It was also reported that ACF suppresses the proliferation of tumor cells (Canellakis and Chen, 1979; Chakrabotry *et al.*, 1984), and the antitumor effects of ACF have been reported in a variety of systems. Although the primary effect of diacridines is the inhibition of RNA, DNA, and protein synthesis, the anti-neoplastic action of acridines is most closely related to their effects on the plasma membrane (Bose *et al.*, 1966; Canellakis and Chen, 1979). Acridines, such as ACF and diacridines, inhibit the cellular transformation by altering the surface membrane of the tumor cell (Chakrabotory *et al.*, 1984). The avid binding of these compounds to the plasma membrane might induce a respiratory deficiency in tumor

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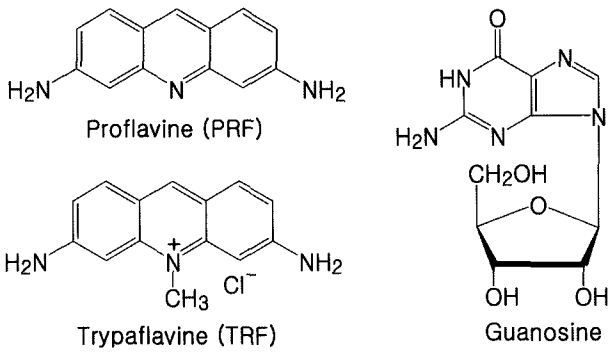


**Fig. 1.** Structural formulae of trypaflavine (TRF), proflavine (PRF), and guanosine. The combination mixture contained acriflavine (ACF; 2:1 mixture of TRF and PRF) and guanosine at a ratio of 1:1.

cells. The membrane binding of ACF leads to the inhibition of protein kinase C through interactions with the regulatory lipid cofactors or the regulatory domain of the protein (Ferey *et al.,* 1986; Roth *et al.,* 1967), which might be one mechanism by which ACF has its antitumor effects (Hannun and Bell, 1988).

Guanosine and guanosine 5'-monophosphate reportedly enhance the antitumor effects of chemotherapeutic agents. They potentiate the anti-tumor activity of 5'-deoxy-5-flurouridine without increasing the toxicity to the host, as observed in cultured cells and animals with xenogrefted tumors (Iigo *et al.,* 1987). Guanosine monophosphate exerts the feedback inhibition of inosine monophosphate (IMP) synthesis, resulting in the inhibition of adenosine monophosphate (AMP) production. As a result, the depletion of AMP might be responsible for the cellular ATP depletion through the inhibition of a feedback product. The accumulation of excess guanosine 5'-monophosphate also inhibits the biosynthesis of ribose phosphate pyrophosphate.

Recently, it was reported that guanosine enhances the antitumor effects of ACF against a variety of cancer cells without causing serious adverse effects (Kim *et al.,* 1997), which has provided a preclinical basis for the potential application of this combination in the treatment of cancer. A 1:1 mixture of ACF and guanosine is currently being evaluated as a possible antitumor agent in preclinical and clinical studies. However, there are no reports of the effects of guanosine on the pharmacokinetics of ACF in any mammalian species. Therefore, this study investigated the effects of guanosine on the pharmacokinetics of TRF and PRF after and intravenous (i.v.) bolus or an intramuscular (i.m.) injection of the mixture in rats. Furthermore, the elimination of TRF and PRF by the biliary and urinary systems and tissue distribution were examined. A high performance liquid chromatography (HPLC)-based method, which has been validated in our laboratory, was used to analyze the TRF and PRF levels in biological samples.

## MATERIALS AND METHODS

### Chemicals and reagents

3,6-Diamino-10-methylacridium chloride (trypaflavine, TRF) and 3,6-diaminoacridine (proflavine, PRF) were obtained from the Taerim Pharmaceutical Co. (Seoul, Korea). Acriflavine (ACF; CAS 8063-24-9), which is a 2:1 mixture of TRF and PRF, was purchased from Aldrich (Milwaukee, WI, U.S.A.). 9-Aminoacridine was supplied by Sigma Chemical Co. (St Louis, MO, U.S.A.). The solvents used in HPLC analysis were of HPLC grade and were filtered and degassed immediately before use. All other chemicals used in this study were of analytical reagent grade.

### Animals

Adult male Sprague Dawley rats, weighing 230-250 g (Sam Tac Co. Ltd., Kyunggi, Korea), were used for the pharmacokinetic studies. The rats were housed in individual metabolic cages during and after administering the drugs. The animals were maintained under a 12 h light/dark cycle with free access to water.

### HPLC analysis of TRF and PRF in biological samples

The TRF and PRF levels were examined by reverse phase HPLC on a Capcell Pak $C_{18}$ column (4.6 mm × 250 mm, 5 μm, Model: UG120, Shiseido Co., Tokyo, Japan) that was interfaced with a Jasco HPLC system consisting of a model PU-980 pump, a model AS-950-10 autoinjector, an UV-VIS detector, and an LC-Net II control Borwin integrator (Jasco Co. Ltd., Tokyo, Japan). The mobile phase contained a mixture of acetonitrile and 0.1% triethylamine solution (17:83, v/v; adjusted to pH 2.2 with phosphoric acid). The flow rate was 1 mL/min. The concentration of TRF, PRF, and 9-aminoacridine (internal standard) in the eluted fractions were monitored spectrophotometrically at a wavelength of 262 nm.

The lower limit of quantitation (LOQ) was defined as the lowest concentration yielding high precision (a coefficient of variation [CV] less than 20%) i.e. an accuracy between 80% and 120% of the theoretical value. The LOQ of either TRF or PRF was found to be 0.05 μg/mL for the plasma, bile, urine, and liver homogenate in five replicate samples. The linearity of the calibration curve was determined by preparing standards of the plasma, bile, urine, and liver homogenates containing TRF or PRF at concentrations ranging from 0.05 to 100 μg/mL. The peak area ratios of the compounds in the internal standards were fitted to straight lines by linear regression. The intra- and inter-day precision and accuracy of the assay was assessed by preparing quality control (QC) samples containing TRF or PRF at concentrations ranging from 0.05 to 100 μg/mL in

each of the biological samples. The intra-day precision of the assay was assessed by calculating the coefficients of variation of an analysis of the QC samples in five replicates, and the inter-day precision was determined through an analysis of the QC samples on five consecutive days. The accuracy of the analysis was determined by comparing the calculated concentration to the known concentration using the calibration curves. The mean recoveries were determined for three concentrations of QC samples (0.05, 1, and 100 μg/mL) with five repeats.

### Analysis of TRF and PRF in the plasma after the i.v. bolus or i.m. injection

Under light sodium pentobarbital anesthesia, the femoral vein and artery of the rats were cannulated with PE-50 polyethylene tubing (Intramedic, Clay Adams, U.S.A.) for both administration and blood sampling, respectively. The combination mixture or ACF dissolved in 0.25 mL of physiological saline was administered through the femoral vein at a dose of 10 mg/kg of the combination mixture (3.33 mg/kg TRF, 1.67 mg/kg PRF, 5 mg/kg guanosine) or at a dose of 5 mg/kg of ACF (3.33 mg/kg TRF, 1.67 mg/kg PRF). For the i.m. dose, 30 mg/kg of the combination mixture (10 mg/kg TRF, 5 mg/kg PRF, 15 mg/kg guanosine) or 15 mg/kg of ACF (10 mg/kg TRF, 5 mg/kg PRF) was administered into the skeletal muscles. Blood (200 μL) was collected from the femoral artery into heparinized tubes at 0.5, 1, 1.5, 2, 3, 5, 7, 10, 15, 20, and 30 min after the i.v. bolus injection, and up to 120 min after the i.m. injection.

The blood samples were centrifuged for 15 min at 1500×g and the plasma was harvested. Immediately after collecting the plasma samples (100 μL), 25 μL of 4 μg/mL 9-aminoacridine (internal standard) was added to each plasma test tube. Methanol containing 1% acetic acid (2.5 mL) was then added to precipitate the proteins and extract the compounds of interest. These mixtures were then vortexed for 15 min and centrifuged for 15 min at 1500×g. The organic layers were withdrawn, dried under a stream of dry nitrogen gas, and reconstituted in 100 μL of the mobile phase for quantitative HPLC analysis.

### Analysis of the biliary and urinary excretion of TRF and PRF

Under light sodium pentobarbital anesthesia, the femoral vein of the rats was cannulated with PE-50 polyethylene tubing for administering the combination mixture or ACF. A catheter (PE-10, Intramedic, Clay Adams, U.S.A.) was then implanted into the bile duct through a small abdominal incision. The blank bile was obtained immediately before the i.v. bolus administration (10 mg/kg mixture or 5 mg/kg ACF) or i.m. administration (30 mg/kg mixture or 15 mg/kg ACF). Bile was collected over a 12 h period after administration. Urine was collected using metabolic cages (Model 3700, Tecniplast, Italia) over a 24 h period after the i.v. or i.m. injection and was stored at -70°C until analysis.

The levels of TRF and PRF in the bile and urine were determined by solid phase extraction (SPE) using a $C_{18}$ column (Bond Elut $C_{18}$ cartridge, 3cc/200 mg, Varian Sample Preparation Products, Harbor City, CA, U.S.A.). The SPE column was preconditioned twice with 2 mL of methanol followed by two applications of 2 mL distilled water before loading the sample. 9-Aminoacridine (25 μL, 4 μg/mL) was added to each test tube containing 100 μL of bile or urine. The mixture was loaded onto the preconditioned SPE column. The column was washed twice with 3 mL of distilled water. TRF and PRF were eluted with 2.5 mL of methanol containing 1% acetic acid. The eluted sample was then dried under a stream of dry nitrogen gas, and reconstituted in 100 μL of the mobile phase for quantitative HPLC analysis.

### Determination of the tissue distribution of TRF and PRF

The rats were sacrificed 15 or 60 min after the i.v. bolus (10 mg/kg mixture or 5 mg/kg ACF) or i.m. (30 mg/kg mixture or 15 mg/kg ACF) injection. The liver, kidney, lung, spleen, stomach, small intestine, large intestine, muscle, and skin were removed immediately, blotted with filter paper, and weighed. Ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose was added to the tissues at a ratio of 1:3 (tissue weight to buffer volume, yielding a 25% homogenate). The tissues were then minced and homogenized using a glass Potter-Elvehjem-type homogenizer with a Teflon pestle. The concentrations of TRF and PRF in the 25% homogenates were measured using the same method as described above for plasma.

### Pharmacokinetic analysis

The TRF or PRF plasma concentration profiles after the i.v. bolus injection of the combination mixture or ACF were analyzed by fitting the data to the following biexponential equation according to a nonlinear least-squares method (MULTI) (Yamaoka *et al.*, 1981):

$$C_p = A \cdot \exp(-\alpha t) + B \cdot \exp(-\beta t).$$

The pharmacokinetic parameters were then calculated as follows:

$$k_{21} = (A\beta + B\alpha)/(A+B),\ k_{el} = \alpha\beta/k_{21},\ k_{12} = (\alpha+\beta) - (k_{21}+k_{el}),$$
$$t_{1/2\alpha} = 0.693/\alpha, \text{ and } t_{1/2\beta} = 0.693/\beta,$$

where $k_{21}$ denotes the transfer rate constant from the peripheral to central compartment, $k_{el}$ represents the elimination rate constant, $k_{12}$ represents the transfer rate constant from central to peripheral compartment, and $t_{1/2\alpha}$ and $t_{1/2\beta}$ represent the plasma half lives at the $\alpha$ and $\beta$

phases, respectively. After injecting the combination mixture i.m., the elimination rate constant (k) of TRF or PRF was calculated from the slope of a line in the terminal phase of plasma disappearance. The plasma half life ($t_{1/2}$) after the i.m. injection was calculated as $t_{1/2} = 0.693/k$ assuming 1st order kinetics. The highest concentration ($C_{max}$) and time to reach the highest concentration ($t_{max}$) were read directly from the time-plasma concentration curves of TRF and PRF.

Non-compartment methods were also used to determine the pharmacokinetic parameters of TRF or PRF after the i.v. bolus or i.m. injection. The area under the plasma concentration-time curve from time zero to infinity (AUC) was calculated from the equation, $AUC = AUC_t + C_t/k$, where $C_t$ is the last quantifiable concentration and k was calculated from the slope of the straight line in the terminal phase of plasma disappearance. The area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration ($AUC_t$) was calculated using a linear trapezoidal approximation. The following parameters were calculated using standard methods:

The total plasma clearance ($CL_t$) = F·Dose/AUC;

The mean residence time (MRT) = AUMC/AUC, where AUMC represents the area under the moment curve; and

The steady-state volume of distribution ($Vd_{ss}$) = $CL_t$·MRT.

The bioavailability (BA, %) of the i.m. dose was calculated from the dose-adjusted ratio of $AUC_{i.m}$ to $AUC_{i.v.}$.

### Statistical analysis

A unpaired Student's t-test was used to compare the two groups. One-way analysis of variance was used to test for any significant differences between multiple groups. A p value < 0.05 was considered significant.

## RESULTS AND DISCUSSION

### Validation of the HPLC method

Fig. 2 show the typical chromatograms of TRF, PRF, and the internal standard (IS) in plasma. Control samples showed no peaks that interfered with the signals for TRF, PRF, or IS. The LOQ was found to be 50 ng/mL for plasma. For the samples prepared in plasma, the mean regression equation for TRF was $y = 1.432x + 0.00536$ ($r^2 = 0.9978$), where y is the peak area ratio of each compound to IS, and x is the concentration. The mean regression equation for PRF in plasma was $y = 1.492x + 0.00845$ ($r^2 = 0.9982$). These equations show significant linearity at concentrations ranging from 0.05 to 100 μg/mL. The mean regression equations for the bile, urine, and tissue homogenates were not significantly different from the equation obtained for plasma.

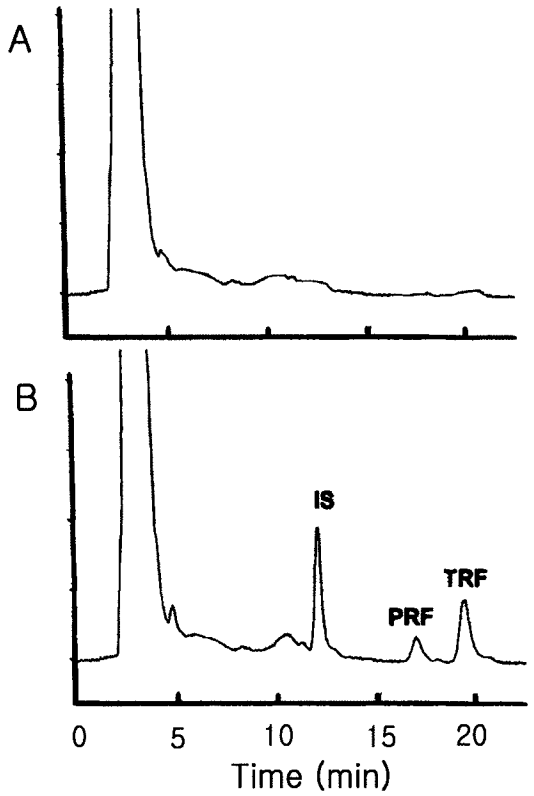


**Fig. 2.** Representative chromatograms of TRF and PRF in plasma. 9-Aminoacridine (25 μL of 4 μg/mL) was added as the internal standard (IS). The upper panel (A) represents a chromatogram of the blank samples, while the lower panel (B) represents a chromatogram of the biological samples after administering the combination mixture. The blank samples show that there are no peaks interfering with the TRF, PRF, and IS signals.

The intra-day accuracies of TRF and PRF never exceeded 14% and had CVs < 11%. In addition, the inter-day accuracies of TRF and PRF never exceeded 12% and had CVs < 10%. This suggests that the present method has satisfactory accuracy, precision, and reproducibility for determining the TRF and PRF levels in plasma, bile, urine, and liver homogenate samples. The mean absolute recoveries of TRF or PRF were > 95.6% and 94.5%, respectively.

### Effects of guanosine on the pharmacokinetics of TRF and PRF

The rats were given i.v. bolus injections of the combination mixture or ACF at doses of 10 or 5 mg/kg. Fig. 3 shows the concentrations of TRF and PRF in rat plasma over time after the administration. TRF and PRF disappeared from the plasma with similar time courses. The concentration of both TRF and PRF decreased rapidly from the plasma within the first 3 min after the i.v. injection (α phase), followed by a slower decrease (β phase). Table I gives a summary of the pharmacokinetic parameters of TRF and

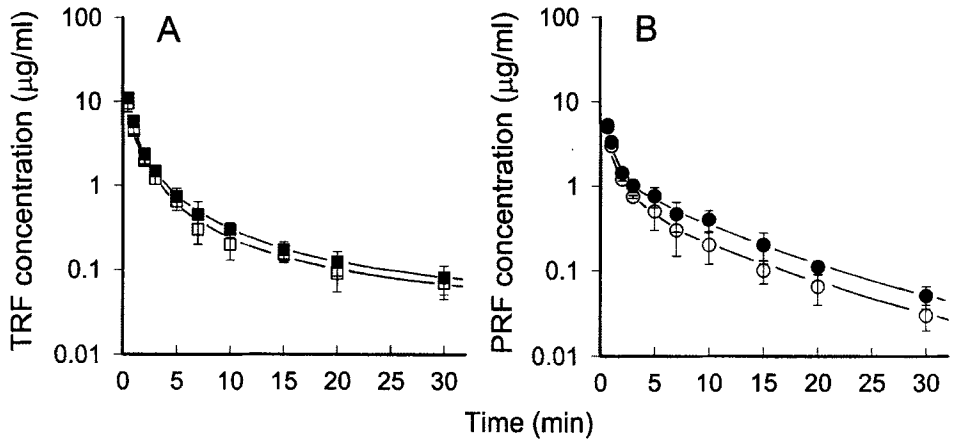


**Fig. 3.** (A) TRF concentrations in the rat plasma over time after i.v. bolus administration of the combination mixture at a dose of 10 mg/kg (■) or ACF at a dose of 5 mg/kg (□). (B) PRF concentrations in the rat plasma over time after i.v. bolus administration of the combination mixture at a dose of 10 mg/kg (●) or ACF at a dose of 5 mg/kg (○). Each point represents the mean ± S.E. of four rats.

**Table I.** Pharmacokinetic parameters of trypaflavine (TRF) and proflavine (PRF) after the i.v. bolus injection of a 1:1 mixture (ACF+guanosine) or ACF at doses of 10 or 5 mg/kg in rats[a]

| Parameter | TRF | | PRF | |
|---|---|---|---|---|
| | ACF+Guanosine | ACF | ACF+Guanosine | ACF |
| A (μg/mL) | 12.6 ± 3.37 | 9.56 ± 5.81 | 7.83 ± 0.935 | 7.06 ± 1.58 |
| B (μg/mL) | 0.602 ± 0.174 | 0.376 ± 0.162 | 1.05 ± 0.285 | 0.982 ± 0.188 |
| α ($min^{-1}$) | 0.848 ± 0.0826 | 0.771 ± 0.137 | 1.22 ± 0.328 | 1.05 ± 0.185 |
| β ($min^{-1}$) | 0.0743 ± 0.0075 | 0.0623 ± 0.0084 | 0.106 ± 0.0274 | 0.105 ± 0.0169 |
| $k_{12}$ ($min^{-1}$) | 0.238 ± 0.0453 | 0.205 ± 0.0532 | 0.543 ± 0.0637 | 0.434 ± 0.0932 |
| $k_{21}$ ($min^{-1}$) | 0.110 ± 0.0237 | 0.089 ± 0.0193 | 0.238 ± 0.0593 | 0.220 ± 0.0634 |
| $k_{el}$ ($min^{-1}$) | 0.575 ± 0.0734 | 0.539 ± 0.0779 | 0.544 ± 0.0531 | 0.501 ± 0.0246 |
| $t_{1/2\alpha}$ (min) | 0.817 ± 0.0648 | 0.899 ± 0.0385 | 0.568 ± 0.0537[b] | 0.660 ± 0.0539[b] |
| $t_{1/2\beta}$ (min) | 9.33 ± 1.64 | 11.1 ± 1.82 | 6.54 ± 0.431[c] | 6.61 ± 0.683[c] |
| AUC (μg · min · $mL^{-1}$) | 23.3 ± 2.25 | 20.3 ± 2.74 | 11.8 ± 2.03 | 10.9 ± 3.69 |
| AUMC (μg · $min^2$ · $mL^{-1}$) | 148 ± 19.3 | 122 ± 15.3 | 82.1 ± 11.7 | 72.2 ± 28.3 |
| MRT (min) | 6.35 ± 0.415 | 6.01 ± 0.623 | 6.96 ± 4.72 | 6.61 ± 9.34 |
| $Vd_{ss}$ (mL/kg) | 909 ± 57.6 | 986 ± 69.3 | 983 ± 62.4 | 1010 ± 57.3 |
| $CL_t$ (mL/min) | 143 ± 9.37 | 164 ± 17.6 | 141 ± 18.3 | 153 ± 13.9 |

[a] Mean ± S.E. (n = 4); The combination mixture was administered at a dose of 10 mg/kg (3.33 mg/kg TRF, 1.67 mg/kg PRF, and 5 mg/kg guanosine) and ACF was administered at a dose of 5 mg/kg (3.33 mg/kg TRF and 1.67 mg/kg PRF) into the femoral veins of rats.
[b] Significantly different from TRF ($p < 0.05$).
[c] Significantly different from TRF ($p < 0.01$).

PRF after the i.v. bolus injection of the combination mixture or ACF. The mean plasma half-lives ($t_{1/2}$) at the α and β phases at a dose of 10 mg/kg mixture were 0.817 and 9.33 min for TRF, and 0.568 and 6.54 min for PRF, respectively. The corresponding values for 5 mg/kg ACF were 0.899 and 11.1 min for TRF, and 0.66 and 6.60 min for PRF. This suggests that guanosine has no effect on the plasma disappearance of TRF or PRF after the i.v. bolus injection of the mixture. The pharmacokinetic parameters were also determined using non-compartment methods. The values of MRT, $Vd_{ss}$ and $CL_t$ of PRF were similar to those of TRF. In addition, the combination mixture showed similar values of MRT, $Vd_{ss}$ and $CL_t$ for both TRF and PRF.

The combination mixture and ACF were then administered i.m. at doses of 30 and 15 mg/kg, respectively, and the concentrations of TRF or PRF in the plasma over time were measured (Fig. 4). The pharmacokinetic parameters were determined using non-compartment analysis and the results are summarized in Table II. PRF was eliminated from plasma more rapidly than TRF after both the i.v. bolus and i.m. injections (Table I, II). This is consistent

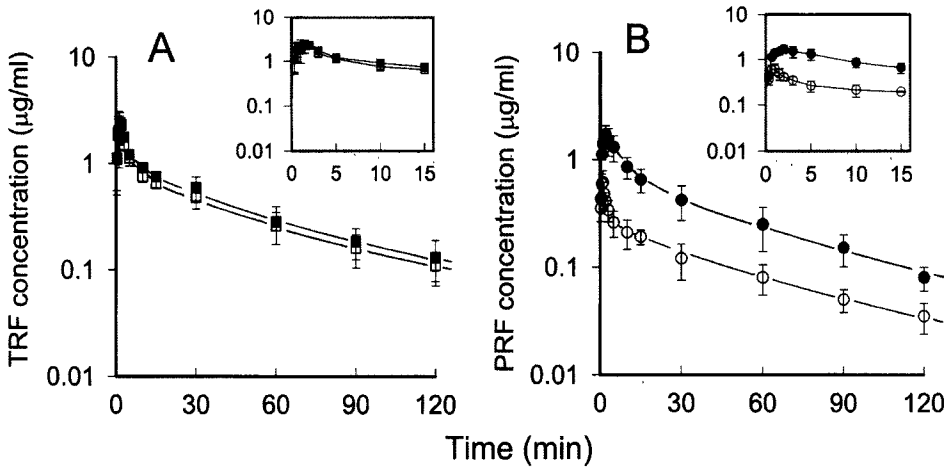


**Fig. 4.** (A) TRF concentrations in the rat plasma over time after the i.m. injection of the combination mixture at a dose of 30 mg/kg (■) or ACF at a dose of 15 mg/kg (□). (B) PRF concentrations in rat plasma over time after the i.m. injection of the combination mixture at a dose of 30 mg/kg (●) or ACF at a dose of 15 mg/kg (○). Each point represents the mean ± S.E. of four rats. Inserts: magnification of the early part of the time course.

**Table II.** Pharmacokinetic parameters of trypaflavine (TRF) and proflavine (PRF) after the i.m. injection of a 1:1 mixture (ACF+guanosine) and ACF at doses of 30 and 15 mg/kg in rats[a]

| Parameter | TRF | | PRF | |
|---|---|---|---|---|
| | ACF+Guanosine | ACF | ACF+Guanosine | ACF |
| $C_{max}$ (μg/mL) | 2.35 ± 0.372 | 2.26 ± 0.437 | 1.73 ± 0.557 | 0.623 ± 0.134 [b] |
| $t_{max}$ (min) | 1.72 ± 0.265 | 1.58 ± 0.432 | 1.63 ± 0.168 | 1.42 ± 0.291 |
| $t_{1/2}$ (min) | 40.1 ± 1.84 | 40.5 ± 1.34 | 36.9 ± 2.93 | 39.4 ± 5.82 |
| AUC ($\mu g \cdot min^2 \cdot mL^{-1}$) | 52.3 ± 5.75 | 46.9 ± 6.53 | 30.1 ± 3.88 | 14.9 ± 4.27 [b] |
| AUMC ($\mu g \cdot min^2 \cdot mL^{-1}$) | 3140 ± 417 | 2736 ± 534 | 1562 ± 274 | 953 ± 125 [b] |
| MRT (min) | 60.3 ± 6.19 | 58.3 ± 4.35 | 51.9 ± 7.18 | 63.9 ± 8.19 |
| $Vd_{ss}$ (L/kg) | 8.62 ± 1.17 | 9.57 ± 2.62 | 7.33 ± 2.36 | 9.78 ± 1.59 |
| $CL_t$ (mL/min) | 144 ± 18.4 | 167 ± 17.3 | 146 ± 18.7 | 151 ± 15.2 |
| BA[d] (%) | 74.9 ± 7.36 | 77.1 ± 9.33 | 85.2 ± 7.37 | 45.6 ± 4.37 [b,c] |

[a] Mean ( S.E. (n = 4); The combination mixture was administered at a dose of 10 mg/kg (3.33 mg/kg TRF, 1.67 mg/kg PRF, and 5 mg/kg guanosine) and ACF was administered at a dose of 5 mg/kg (3.33 mg/kg TRF and 1.67 mg/kg PRF) into the femoral veins of rats.
[b] Significantly different from AG60 ($p < 0.01$)
[c] Significantly different from TRF ($p < 0.01$)
[d] Bioavailability (BA, %) was calculated the following equation: BA (%) = (AUCi.m./AUCi.v.) × (Dosei.v./Dosei.m.) × 100.

with the observations reported for channel catfish (Plakas *et al.* 1998). However, the i.m. route leads to a prolongation of TRF or PRF in the plasma compared with the i.v. route, with an approximately 5-fold larger $t_{1/2}$ (36-41 min) (Table II). This indicates a 74.9% and 85.0% bioavailability of TRF and PRF after injecting the combination mixture i.m.. After the i.m. injection, the $C_{max}$ and $t_{max}$ at a dose of 30 mg/kg mixture were 2.35 μg/mL and 1.72 min for TRF, and 1.73 μg/mL and 1.63 min for PRF, respectively. The corresponding values for 15 mg/kg ACF were 2.26 μg/mL and 1.58 min for TRF, and 0.623 μg/mL and 1.42 min for PRF, respectively. The $t_{max}$ of PRF was comparable to that of TRF, and guanosine had no effect on the $t_{max}$ of either PRF or TRF. However, the $C_{max}$ of PRF after administering ACF was 1/3 lower than that after injecting the combination mixture i.m.. This suggests that guanosine leads to a prolongation of the plasma levels of PRF. As a result, the AUC or bioavailability (BA) of PRF after ACF administration was 50% that after administering the combination mixture i.m.. In contrast, there was no significant difference in the MRT, $Vd_{ss}$ and $CL_t$ values between the combination mixture and ACR groups.

## Effects of guanosine on the biliary and urinary excretion of TRF and PRF

Fig. 5 shows the cumulative amounts of TRF or PRF that were excreted into the bile after administering the combination mixture or ACF by an i.v. bolus (10 or 5 mg/

kg) or i.m. (30 or 15 mg/kg). TRF or PRF were fully excreted in the bile within 9-10 h after administration. The cumulative amounts of TRF excreted in the bile over 12 h were 204 μg/kg after the i.v. bolus injection of 10 mg/kg of the mixture, and 318 μg/kg after the i.m. injection of 30 mg/kg of the mixture. These values represent approximately 6.12% and 4.25% of the TRF that had been administered i.v. or i.m., respectively. The cumulative amounts of PRF excreted in the bile over 12 h after administering the combination mixture were calculated to be approximately 7.8% of the PRF that had been administered i.v. and 3.9% of the amount administered i.m.. After the i.v. bolus injection, the cumulative amounts of TRF or PRF after administering the ACF were comparable to those after administering the mixture (Fig. 5A). However, after the i.m. injection, the cumulative amounts of TRF or PRF after administering the ACF administration were approximately 1.25 times higher than those after administering the mixture (Fig. 5B).

Fig. 6 shows the cumulative amounts of TRF or PRF that had been excreted into the urine after the i.v. bolus or i.m. administration of the combination mixture or ACF. The cumulative amounts of TRF excreted in the urine over 24 h were 163 μg/kg after the i.v. bolus injection of 10 mg/kg mixture, and 277 μg/kg after the i.m. injection of 30 mg/kg mixture. These values represent approximately 4.9% and

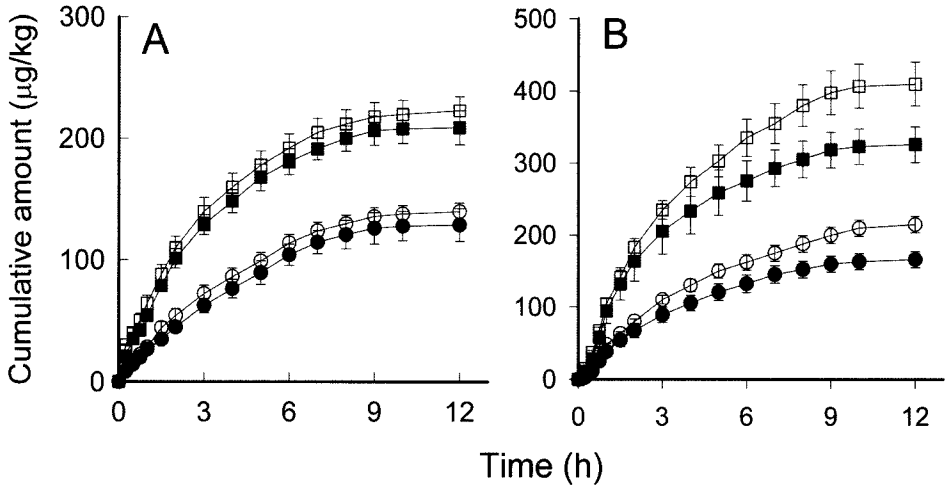


**Fig. 5.** Cumulative biliary excretion of TRF and PRF after the i.v. bolus injection of the combination mixture at a dose of 10 mg/kg or ACF at a dose of 5 mg/kg (A), and the i.m. injection of the combination mixture at a dose of 30 mg/kg or ACF at a dose of 15 mg/kg (B). Each point represents the mean ± S.E. of four rats. Symbols: (A) (□) TRF after i.v. dose of ACF (5 mg/kg); (■) TRF after i.v. dose of the mixture (10 mg/kg); (○) PRF after i.v. dose of ACF (5 mg/kg); (●) PRF after i.v. dose of the mixture (10 mg/kg), (B) (□) TRF after i.m. dose of ACF (15 mg/kg); (■) TRF after i.m. dose of the mixture (30 mg/kg); (○) PRF after i.m. dose of ACF (15 mg/kg); (●) PRF after i.m. dose of the mixture (30 mg/kg).

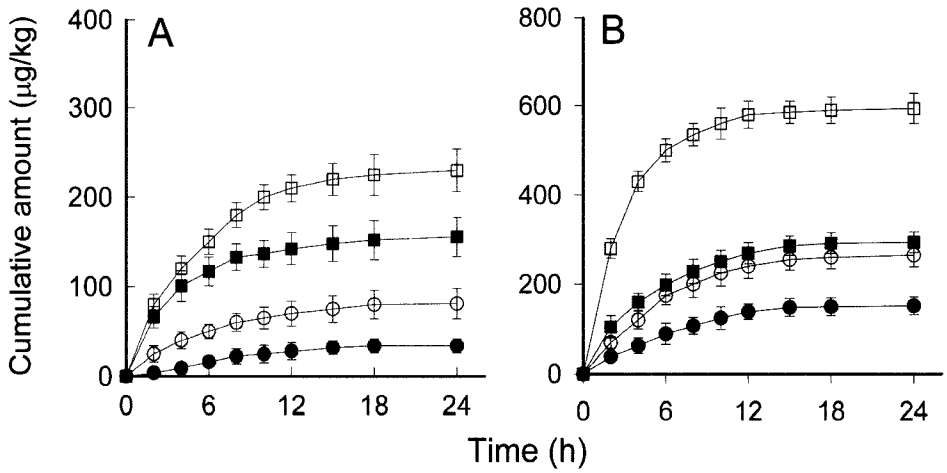


**Fig. 6.** Cumulative urinary excretion of TRF and PRF after the i.v. bolus injection of the combination mixture at a dose of 10 mg/kg or ACF at a dose of 5 mg/kg (A), and the i.m. injection of the combination mixture at a dose of 30 mg/kg or ACF at a dose of 15 mg/kg (B). Each point represents the mean ± S.E. of four rats. Symbols: (A) (□) TRF after i.v. dose of ACF (5 mg/kg); (■) TRF after i.v. dose of the mixture (10 mg/kg); (○) PRF after i.v. dose of ACF (5 mg/kg); (●) PRF after i.v. dose of the mixture (10 mg/kg), (B) (□) TRF after i.m. dose of ACF (15 mg/kg); (■) TRF after i.m. dose of the mixture (30 mg/kg); (○) PRF after i.m. dose of ACF (15 mg/kg); (●) PRF after i.m. dose of the mixture (30 mg/kg).

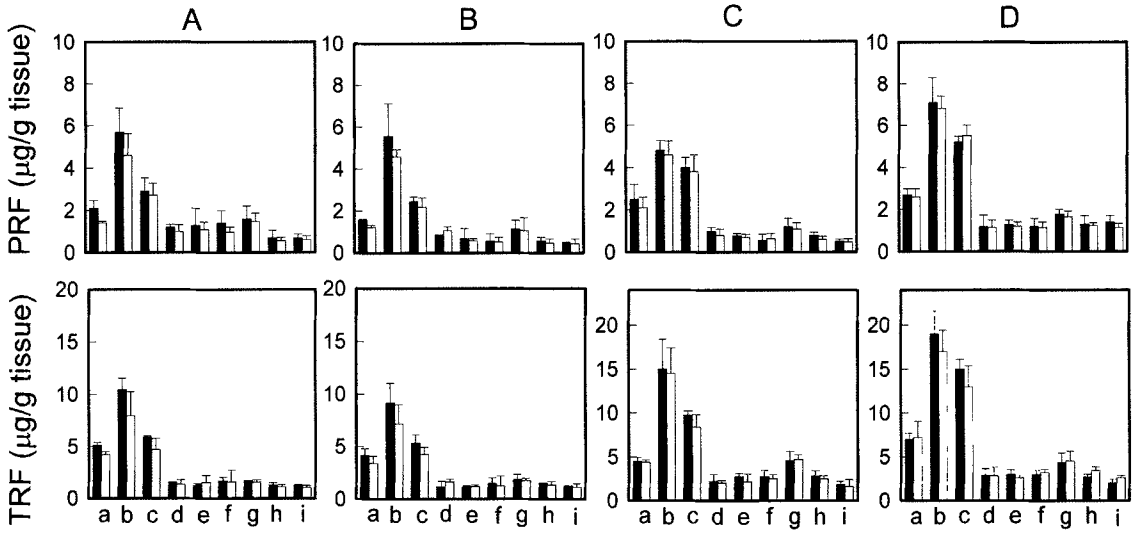


**Fig. 7.** Tissue distribution of PRF (upper panel) and TRF (lower panel) at 15 or 60 min after the i.v. bolus or i.m. injection of the combination mixture of ACF and guanosine (■) and ACF (□). (A) 15 min after the i.v. injection of 10 mg/kg mixture or 5 mg/kg ACF; (B) 60 min after the i.v. injection of 10 mg/kg mixture or 5 mg/kg ACF; (C) 15 min after the i.m. injection of 30 mg/kg mixture or 15 mg/kg ACF; (D) 60 min after the i.m. injection of 30 mg/kg mixture or 15 mg/kg ACF. Keys: (a) liver; (b) kidney; (c) lung; (d) small intestine; (e) large intestine; (f) stomach; (g) spleen; (h) muscle; (i) skin.

3.70% of the TRF that had been administered i.v. or i.m., respectively. The cumulative amounts of PRF excreted in the urine over a 24 h period after administering the mixture were calculated to be approximately 2.1% and 3.6% of the PRF administered i.v. and i.m., respectively. After the i.v. injection, the cumulative amounts of TRF or PRF after administering the ACF were approximately 1.5 and 2.4 times higher than those after administering the mixture, respectively (Fig. 6A). Moreover, after i.m. administration, the cumulative amounts of TRF or PRF after administering the ACF were approximately 2.1 and 1.8 times higher than those after administering the mixture, respectively (Fig. 6B).

Effects of guanosine on the biliary and urinary excretion of TRF and PRF were more pronounced after the i.m. injection compared with those after the i.v. bolus injection. With the exception of the biliary excretion of TRF or PRF after the i.v. bolus injection (Fig. 5A), guanosine reduced the biliary and urinary excretion of TRF or PRF by 1.25-2.1 fold after the i.v. bolus or i.m. injection (Fig. 5B, Fig. 6). The combination mixture increased the BA of PRF by approximately 2 folds after the i.m. injection whereas guanosine did not significantly alter the plasma levels of TRF after the i.m. injection. However, more study will be needed to determine the relationship between the plasma levels and the biliary or urinary excretion of TRF or PRF.

### Effects of guanosine on the tissue distribution of TRF and PRF

Fig. 7 shows the distribution of TRF or PRF in various tissues 15 and 60 min after the i.v. bolus or i.m. injection of the combination mixture or ACF. TRF and PRF were found at significant levels in all tissues examined but showed the highest concentration in the kidney. The concentration of TRF and PRF in the kidney 60 min after the i.v. bolus injection of 10 mg/kg AG60 were comparable to the concentration in the plasma shortly after the i.v. bolus injection (Fig. 3). Moreover, the concentrations of TRF and PRF in the kidney 60 min after the i.m. injection of 30 mg/kg of the mixture were much higher than its $C_{max}$ (Fig. 4, Table II). However, the concentrations of TRF and PRF in all tissues examined were similar in the combination mixture and ACR groups.

TRF and PRF were widely distributed in the tissues of channel catfish after an i.v. injection, and the tissue concentrations were maintained at high levels for a long period of time (Plakas *et al.*, 1998). The TRF and PRF concentrations in the tissues were relatively stable 60 min after the i.v. bolus or i.m. injection of the combination mixture but decreased to below the quantifiable limit by 48 h (data not shown). The slow elimination of these drugs from the tissues might be caused by their slow excretion to the bile or urine (Figs. 5, 6). In addition, the high affinity or binding in these tissues may prolong the tissue distribution of TRF or PRF.

## CONCLUSION

The antitumor properties of a 1:1 mixture of ACF and guanosine are currently undergoing preclinical tests to determine their suitability for clinical trials (Kim *et al.*, 1997). Therefore, this study examined the effects of guanosine on the pharmacokinetics of ACF (TRF and PRF) after i.v. bolus and i.m. injections of the combination

mixture into rats. It was found that TRF and PRF were rapidly cleared from the blood and transferred to the tissues after the i.v. bolus or i.m. of the combination mixture. Both TRF and PRF were most highly concentrated in the kidneys after the i.v. bolus or i.m. injection, followed by the slow excretion to the bile or urine. Guanosine had no effect on the plasma disappearance of TRF or PRF after the i.v. bolus injection. However, guanosine prolonged the plasma levels of PRF after the i.m. injection of the combination mixture, resulting in a 2-fold increase in the bioavailability (BA) of PRF. Unexpectedly, the concentrations of TRF and PRF in all tissues examined were similar in the groups given the combination mixture and ACR. However, guanosine prolonged the urinary excretions of both TRF and PRF. These effects of guanosine on the plasma disappearance or urinary excretion of TRF and PRF might account for the enhanced antitumor effects of ACF. However, more study will be needed to confirm this potential mechanism.

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