Original Contribution

Effect of Hepatic Hyperthermia on Plasma Concentration-Time Curve of Pirarubicin after Hepatic Arterial Infusion in Patients with Primary or Secondary Liver Cancer

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Abstract : Intravenous plasma concentration-time curve and AUC of pirarubicin, which is metabolized mainly in the liver, infused in the hepatic artery with or without hyperthermia is compared. One patient with hepatocellular carcinoma and 4 cases with metastatic liver cancer were enrolled. Pirarubicin (THP-ADR) (15mg/m²) was infused in proper hepatic artery in 20 minutes from implanted catheter. Intravenous plasma concentration of the drug was measured immediately after injection, 0.5, 1, 3 and 6 hours after injection respectively. Three weeks later, the same amount of the drug was injected to the same patient concomitantly with hyperthermic treatment of the liver using Thermotron RF8 and the drug concentration was measured. Changes of WBC, platelets counts, total bilirubin and AST (GOT) after pirarubicin injection with or without hyperthermia were also compared. Mean value of AUC (HR×μg/ml) of the injected pirarubicin without and with hyperthermia were 0.068 ± 0.022 and 0.062 ± 0.023 respectively. No significant difference of AUC between hepatic injection alone and injection during hyperthermia was seen by paired *t*-test (*p* = 0.563). No significant difference was seen in WBC, platelets counts, total bilirubin and AST between pirarubicin injection with and without hyperthermia. It was suggested that toxicity of pirarubicin injected in hepatic artery would not increase even if combined with hyperthermic treatment of the liver.

Introduction

Metastatic liver cancer or advanced hepatocellular carcinoma were hardly curable disease. To treat such patients, intra-arterial infusion of anti-neoplastic agents is attempted. Compared to conventional intravenous administration, hepatic intra-arterial infusion therapy of anti-neoplastic agent, pirarubicin

(THP-ADR), is accompanied with reduction of area under the concentration-time curve (AUC)¹⁾, increase of tumor concentration of pirarubicin²⁾. Intra-hepatic arterial infusion is effective modality to treat these patients³⁾.

Hyperthermia is another modality used for cancer treatment⁴⁾. Hyperthermia at 43-44°C shows cytotoxicity to mammalian cells. p53 dependent and independent apoptosis was caused by hyperthermia^{5) 6)}. Increase of intracellular Ca²⁺ concentration is also an important factor of apoptosis induced by hyperthermia⁷⁾. Hyperthermia is reported to enhance cellular toxicity of some anti-neoplastic agents by increasing intra-cellular concentration of the drugs^{8) 9)}, enhancing immunological mechanism of the patients¹⁰⁾ or modulating the expression of some cell cycle regulatory proteins¹¹⁾. Some anti-neoplastic agents enhance cytotoxic effect of hyperthermia by reducing the accumulation of heat shock proteins induced by hyperthermia¹²⁾. Thus, combination therapy of hyperthermia and intra-hepatic arterial infusion of some anti-neoplastic agents is effective modality to treat these patients.

However, such combination therapy may increase hepatic toxicity of the drugs. To establish such combination treatment, it is essential to estimate whether the combination therapy is related with increased hepatic toxicity or not. In this report, we have measured the time course of intravenous concentration of pirarubicin infused in hepatic artery with or without hyperthermia and AUC of the pirarubicin with or without hyperthermia was compared. Changes of WBC counts, platelet counts, total bilirubin, AST (GOT) were also compared between the infusion with or without hyperthermia.

Patients and methods

One patient with primary hepatocellular carcinoma and 4 patients with metastatic liver cancer were enrolled in this study. Primary tumors of the four metastatic liver tumors were as follows: colon cancer, gastric cancer, duodenal cancer and cancer of extrahepatic bile duct. Four patients were male and one was female. Patients' age ranged from 58 to 73 years old (mean 65.2 years old). Performance status of these patients were 2 or less in WHO scale. Surgical resection or transarterial embolization therapy was not indicated for these patients. Informed consents were obtained from all patients.

Arterial infusion therapy

Angiocatheter were inserted from right femoral artery to proper hepatic artery under fluoroscopy. Tip of the catheter were fixed in the artery and proximal end of the catheter were connected to reservoir implanted in subcutaneous tissue of right groin. Pirarubicin was infused from the catheter into proper hepatic artery via the reservoir.

Fifteen mg/m² of pirarubicin was infused from the catheter in 20 min. Venous blood samples were taken from the patient immediately after the arterial infusion, 0.5, 1, 3 and 6 hours after the infusion. Three weeks after the infusion, the same patient received the injection of the same amount of pirarubicin from the implanted catheter in 20 minutes concomitantly with hyperthermic treatment of the liver. Venous samples were taken at the same time course after the infusion from the patient.

Hyperthermia

Intra-hepatic tumors of these patients were treated also by regional hyperthermia using 8 MHz capacitive heating. Thermotron RF8 (Yamamoto Vinyter Co., Ltd., Osaka, Japan) was used at hyperthermia. Two opposed circular electrodes with 25 cm diameter covered by water pad were attached at the upper abdomen and back of the patients. An overlay of water was placed between the electrodes and skin to avoid excessive heating of the skin and subcutaneous tissue. Electromagnetic power up to 1300W was applied between the electrodes. Heating time ranged between 40-60 min.

Measurement of pirarubicin concentration

Immediately after venous blood sampling, centrifuged, and the supernatant was frozen at -80°C until measurement. Plasma concentration of pirarubicin was measured by HPLC assay¹³⁾. AUC (area under the concentration-time curve, $\text{HR} \times \mu\text{g/ml}$) up to 6 hours was calculated from plasma concentration-time curve of the pirarubicin. The AUCs of pirarubicin with and without hyperthermia were compared. Significance of the differences of AUCs between with and without hyperthermia was estimated by paired *t*-test. Changes of WBC counts, platelets counts, total bilirubin, AST between before and 2 weeks after arterial infusion of pirarubicin was calculated and the significance of the differences between arterial infusion without and with hyperthermia was estimated by paired *t*-test.

Fig. 1. Plasma concentration-time curve of pirarubicin (THP-ADR) after hepatic arterial infusion in 5 patients. Intra-venous concentration of pirarubicin was measured by HPLC assay immediately after the infusion, 0.5, 1, 3 and 6 hours after the infusion. Ordinate indicates plasma concentration of pirarubicin at $\mu\text{g/ml}$. Abscissa indicates time course (hours) after arterial infusion. Solid circles indicate values without hyperthermia while solid squares indicate values with hyperthermia.

Results

Intra-venous plasma concentration-time curves of pirarubicin after intra-hepatic arterial infusion without and with hyperthermia in each patient were shown in Fig. 1. Mean value of AUC ($\text{HR} \times \mu\text{g}/\text{ml}$) of the injected pirarubicin without and with hyperthermia were 0.068 ± 0.022 and 0.062 ± 0.023 respectively. No significant difference of the AUC between hepatic arterial injection alone and injection during hyperthermia was seen by paired *t*-test ($p = 0.563$) (Fig. 2).

Changes of WBC counts between before the arterial injection of pirarubicin and 2 weeks after the injection were calculated by WBC value before injection minus WBC value 2 weeks after the injection. The changes of WBC values were compared between injection with and without hyperthermia (Fig. 3a). Mean changes of WBC counts ($\times 10^3/\text{mm}^3$) between before and after the injection without and with hyperthermia were -1.66 ± 1.56 and -0.68 ± 1.44 respectively. No significant difference was seen by paired *t*-test ($p = 0.809$). Mean changes of platelets counts ($\times 10^3/\text{mm}^3$) after the injection without and with hyperthermia were -30 ± 64.4 and -2.4 ± 15.7 respectively. No significant difference was seen by paired *t*-test ($p = 0.772$) (Fig. 3b). Changes of total bilirubin (mg/dl) between before the arterial injection of pirarubicin and 2 weeks after the injection was compared (Fig. 3c). Mean changes of total bilirubin between before and after the injection without and with hyperthermia were 0.02 ± 0.20 and

Fig. 2. Area under the concentration-time curve (AUC) ($\text{HR} \times \mu\text{g}/\text{ml}$) after hepatic arterial infusion of pirarubicin without and with hyperthermia in 5 patients. No significant difference was seen between the AUCs with and without hyperthermia by paired *t*-test ($p = 0.563$).

Fig. 3a. Changes of WBC counts ($\times 10^3/\text{mm}^3$) after arterial infusion of pirarubicin without and with hyperthermia. Values of WBC counts before the infusion minus after the infusion were compared between the infusion without and with hyperthermia in each patient. No significant difference was seen by paired *t*-test ($p = 0.809$).

Fig. 3b. Changes of platelets counts ($\times 10^3/\text{mm}^3$) after arterial infusion of pirarubicin without and with hyperthermia. Values of platelets counts before the infusion minus after the infusion were compared between the infusion without and with hyperthermia in each patient. No significant difference was seen by paired *t*-test ($p = 0.772$).

Fig. 3c. Changes of total bilirubin (mg/dl) after arterial infusion of pirarubicin without and with hyperthermia. Values of total bilirubin before the infusion minus after the infusion were compared between the infusion without and with hyperthermia in each patient. No significant difference was seen by paired *t*-test ($p = 0.25$).

-0.16 $\pm$ 0.46 respectively. No significant difference was seen by paired *t*-test ($p = 0.25$). Mean changes of serum AST (IU/l) between before and after the injection without and with hyperthermia were 9.2 $\pm$ 16.1 and -17.6 $\pm$ 22.4 respectively. AST values tend to decrease after the infusion with hyperthermia, but the difference was not significant by paired *t*-test ($p = 0.095$). At least, increase of AST value after the infusion with hyperthermia was not observed (Fig. 3d).

Discussion

Hyperthermia was attempted to treat primary or secondary liver tumors in some institute and some anti-tumor effect was reported ^{14) 15)}. Pirarubicin, in combination with 5Fu and folinic acid, is reported to be effective to treat some liver tumor ¹⁶⁾. The combination therapy of hyperthermia and intra-hepatic infusion of pirarubicin might be effective in the treatment of liver tumors.

Pirarubicin, 4'-O-tetrahydropyranyl-adriamycin (THP-ADR), is developed to reduce cardiotoxicity of adriamycin ^{17) 18)} and has equal anti-tumor activity with adriamycin ¹⁹⁾. Pirarubicin is metabolized mainly in the liver and excreted mostly in bile ^{20) 21)}. When pirarubicin is administered from hepatic artery to treat hepatic tumor, the agent is extracted from liver and related with reduced systemic toxicity ²²⁾

Fig. 3d. Changes of AST (GOT) (IU/l) after arterial infusion of pirarubicin without and with hyperthermia. Values of AST before the infusion minus after the infusion were compared between the infusion without and with hyperthermia in each patient. AST values tend to decrease after the infusion with hyperthermia, but the difference was not significant by paired *t*-test ($p = 0.095$). At least, increase of AST after the infusion with hyperthermia was not observed.

toxicity increase or not by combination therapy, the AUC of pirarubicin with and without hyperthermia was compared. Though the number of patients was small, no significant increase in AUC was observed when combined with hepatic hyperthermia. The changes in WBC, platelets counts, total bilirubin, serum AST after hepatic infusion of pirarubicin were also compared between infusion alone and infusion combined with hepatic hyperthermia. No significant differences in WBC and platelets counts were seen when combined with hyperthermia. No significant increase in total bilirubin and AST were seen in the combination therapy. The results are, however, not definitive because the observation period to assess the change in total bilirubin and AST is too short. Nevertheless, the systemic toxicity may not increase even in combination therapy with hepatic hyperthermia because the AUC of pirarubicin was not increased in the combination therapy. Further clinical study of combination therapy with hepatic intra-arterial infusion of pirarubicin and hepatic hyperthermia in the treatment of advanced primary and metastatic liver cancer is warranted.

and increase in tumoral drug distribution³⁾. Median AUC of intravenous administration of pirarubicin is 4 times higher than that of intra hepatic arterial infusion¹⁾.

On the other hand, it is reported that mild hyperthermia enhances cytotoxic effect of pirarubicin²³⁾. In the treatment of deep-seated tumor by hyperthermia, it is difficult to obtain high intratumoral temperature, i. e., 43°C or more. So, pirarubicin is useful agent in combination treatment with hyperthermia in deep-seated tumor such as intrahepatic tumor because cytotoxicity of pirarubicin is enhanced even in mild hyperthermia. In this report, we have attempted to treat patients with primary or metastatic liver cancer by combination of intra hepatic arterial infusion of pirarubicin and hepatic hyperthermia. Unfortunately, no tumor response was obtained neither after pirarubicin injection alone nor after combination therapy of hyperthermia and pirarubicin injection in all five cases in this trial. Small administration dose (15mg/m²) of pirarubicin may be the reason. To repeat the arterial infusion therapy alone or in combination with hyperthermia will also be necessary to obtain tumor response.

To assess whether hepatic and/or systemic

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原発性、転移性肝癌におけるピラルビシン肝動脈内 投与後の静脈血中薬剤濃度の推移に及ぼす温熱療法の影響

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要 旨： 肝癌に対し肝動脈内に留置したカテーテルからピラルビシンを注入し、静脈内濃度を測定し、温熱併用時と非併用時とで、その AUC を比較した。原発性肝癌 1 例、転移性肝癌 4 例にピラルビシン (15mg/m²) を固有肝動脈に留置したカテーテルから 20 分で注入し、注入直後、30 分、1、3、6 時間後の血中薬剤濃度を測定した。3 週間後サーモトロン RF8 による温熱療法同時併用下に同様に薬剤を注入し、濃度を測定した。温熱非併用、併用時のピラルビシン動注後の AUC (HR×μg/ml) の平均値は各々 0.068 及び 0.062 で、温熱併用、非併用時の AUC に paired *t*-test において有意差は認めなかった (*p* = 0.563)。白血球数、血小板数、総ビリルビン、AST (GOT) 値の変化にも温熱併用、非併用時で有意差は認めなかった。ピラルビシンを肝動脈から注入した時の毒性は温熱を併用しても増強しないことが示唆された。