Retention of Perfluorochemicals in Tumor-Bearing Rats

Yoshio TSUDA, Ryoichiro MURASHIMA, Kouichi YAMANOUCHI, Yasuo UEDA, and Kazumasa YOKOYAMA

The Green Cross Corporation, Shodai-Ohtani-2-1180-1, Hirakata, Osaka 573, Japan

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In order to study the elimination rate of perfluorochemical (PFC) emulsion from the blood and the PFC contents in the main organs of the tumor-bearing animals, Wistar strain rats were intramuscularly implanted with Walker 256 carcinosarcoma and 9L glioma cells were inoculated into the femoral muscle of Fischer rats. After 7 d the Wistar rats received an injection of 4 g PEC/kg of a PFC emulsion. The Fischer rats received a similar injection after 11 d. The rats injected with the PFC emulsion were periodically sacrificed for a week. There was no significant difference in PFC contents in the blood and main organs between normal rats and the tumor-bearing rats with either Walker 256 carcinosarcoma or 9L glioma. The PFC contents in Walker tumor were about 10 times as much as those of 9L tumor. The inoculated tumors did not substantially affect the PFC retention in the blood and the accumulation of PFC in main organs after intravenously injected with the PFC emulsion.

Keywords — perfluorochemical; distribution; perfluorochemical emulsion; Walker carcinosarcoma; 9L glioma; Wistar rat; Fischer rat

Introduction

One of the approaches to blood substitutes is to utilize perfluorochemicals (PFC) which have a high oxygen carrying capacity in solution, as exemplified by the mixed PFC emulsion, Fluosol-DA, 20% (Fluosol). Fluosol was first infused into human volunteers and patients.^{1,2)} The initial aim of Fluosol had been to save the life of the patient who had lost great quantities of the circulating blood. The limitation of the PFC emulsion, however, such as less oxygen solubility compared to blood and the necessity of high oxygen concentrations in the inspired air, led us to pursue other indications except the hemorrhage.³⁾ Such potential indications of the PFC emulsion are, for instance, an oxygen-carrying solution for a percutaneous transluminal cardioangioplasty to the patients with the cardiovascular stenosis⁴⁾ and a sensitizer for a radiation therapy and chemotherapy in the oncology field.^{5,6)}

Teicher demonstrated that the radiation under the carbogen (95% oxygen and 5% carbon dioxide) inspiration after the Fluosol infusion gave better results in reduction of the tumor growth compared to the radiation alone or with the carbogen inspiration.⁶⁾ The PFC particles

are presumed to carry sufficient oxygen to the hypoxic tumor cells enough to increase the sensitivity against radiation.⁷⁾ Following preclinical studies, the clinical trials with Fluosol have been done for patients having carcinoma in the head and neck.⁸⁾

It was demonstrated by Sasai *et al.* that the efficacy of the PFC emulsion was dependent upon the type of tumor, when it was used in four experimental solid tumors of mice.⁹⁾ It is of particular interest to us to clarify the basis for tumor-dependency. In order to determine this from the PFC profile in blood and the PFC accumulation in tumors, two kinds of tumors implanted to the rats intramuscularly were used for our experiments; Walker 256 carcinosarcoma and 9L glioma. With regard to 9L glioma, Kuwamura and Korosue showed the synergistic effect of a PFC emulsion in radiation therapy.^{10,11)} However, the efficacy study using Walker tumor has not been reported, but Klubes *et al.*¹²⁾ examined the effect of Fluosol on the blood flow in the Walker tumor implanted s.c., and showed that Fluosol did not increase intratumoral blood flow. Hence, treatment with the PFC emulsion might not enhance the action of radiation against the rat Walker tumor, since the tumor blood flow is an important deter-

minant of the efficacy in cancer therapy.¹²⁾

We have examined the behavior of a PFC emulsion in rats having two different kinds of tumors to answer simple but important questions whether tumor-bearing animals alter the PFC blood level and the distribution of PFC in organs in connection with the tumor-dependent efficacy.

Materials and Methods

A PFC emulsion used in this study was prepared with a Gaulin homogenizer (Gaulin, U.S.A.).¹³⁾ As shown in Table I, the composition of the PFC emulsion (PFC-E) are 25% PFC [17.5% perfluorodecalin (FDC) and 7.5% perfluorotri-*n*-propylamine (FTPA)], 3.2% Pluronic F68, 1.0% glycerol, and 0.5% yolk phospholipids. PFC-E formulated with neither balanced salts nor hydroxyethyl starch was used for this study. Prior to infusion, NaCl was added to PFC-E to provide isotonicity.

Experiment with Walker Tumor — Eighty male Wistar rats weighing 200 to 250 g were divided in two groups. The rats of one group were implanted with 1×10^6 of Walker 256 carcinosarcoma cells into the left femoral muscle. Five days after implantation, when the tumor weight reached about 1 g, all of the tumor-bearing rats and the untreated rats (controls) were injected with PFC-E at a dose of 4 g PFC/kg intravenously. Of ten animals in the 80 rats, 5 tumor-bearing rats and the 5 normal rats, were used for collecting blood samples periodically. A half milliliter of blood was collected from the jugular vein 1, 3, 6, 24 and 48 h after injection.

Seventy rats, which were different from the animals giving the blood samples, were sacrificed

1, 3, 6, 24, 48, 72 and 168 (1 week) h after the injection of PFC-E. The liver, spleen, lung, heart, kidney, adipose tissue, bone marrow, right femoral muscle and the tumor were excised, and about 0.5 g of the sample were weighed and homogenized by a Biotron homogenizer for measuring PFC contents in each of the organs.

Experiment with 9L Glioma — 40 of the 80 male Fischer rats weighing 200 to 250 g were implanted with 1×10^6 of 9L glioma cells into the left femoral muscle. Eleven days after the implantation, when the tumor weights were about 0.5 g, the tumor-bearing rats and the normal rats were injected with PFC-E at a dose of 4 g PFC/kg. A half milliliter of blood was collected from the jugular vein periodically, as described for the experiment with the Walker tumor. The rest of the rats injected with PFC-E were sacrificed at several intervals after injection as described above, and the PFC contents were determined on the homogenates of 0.5 g of each organs.

Determination of PFC — After extracting PFC from the tissue, PFC was determined by gas chromatography, according to the reported method.¹⁴⁾ A brief outline of the method is as follows: a half milliliter of the blood or the tissue homogenates was placed in a test tube, to

Fig. 1. Blood Levels of PFC after Intravenous Injection of PFC-E in Normal Wistar Strain Rats (○) and Walker 256 Carcinosarcoma-Bearing Wistar Rats (●)

TABLE I. Composition of PFC Emulsion

Ingredients	Concentration (w/w%)
Perfluorodecalin	17.5
Perfluorotri- <i>n</i> -propylamine	7.5
Pluronic F68	3.2
Glycerol	1.0
Yolk phospholipids	0.5
Sodium chloride	0.78

which 3 ml ethanol, 3 ml trichlorotrifluoroethane and 4 ml water were added. After vigorous shaking, the mixture was separated into two phases by centrifugation at 3000 rpm for 10 min. One microliter of the lower trichlorotrifluoroethane layer was injected into a gas chromatograph equipped with an OV-17 GC column, 2 m length $\times$ 4 mm i.d., and FDC and FTPA were separately determined. PFC contents were expressed as sum of FDC and FTPA.

Results

Differences in PFC Blood Levels

The Wistar rats implanted with Walker tumor and the normal rats were injected with 20 ml/kg of PFC-E. Figure 1 shows the elimination of injected PFC from the circulating blood. The mean PFC levels in the blood of 5 animals decreased quickly, to about 10% at 24 h and lowered to 1.0 mg/ml after 48 h. No significant difference was observed between the tumor-bearing rats and the normal rats throughout the observation

Fig. 2. Blood Levels of PFC after Intravenous Injection of PFC-E in Normal Fischer Rats (○) and 9L Glioma-Bearing Fischer Rats (●)

period.

The elimination curves of PFC in the Fischer rats are shown in Fig. 2. The Fischer rats tended to maintain the PFC blood levels longer than the Wistar rats. The PFC levels of the Fischer rats, either tumor-bearing or normal, decreased to

Fig. 3. PFC Contents in Main Organs after Injection of PFC-E in Walker 256 Carcinosarcoma-Bearing Rats (a) and Normal Rats (b)

—▼—, liver; —■—, spleen; —○—, lung; —□—, bone marrow; —△—, adipose tissue; —●—, tumor; —●—, blood; —×—, muscle.

20% of the initial level at 24 h but could be detected at 48 h. Like the Wistar rats, the Fischer rats showed that the implanted tumor did not affect the intravascular behavior of PFC particles.

When the apparent elimination rates were calculated, the half lives of the Wistar and the Fischer rats were approximately 7 and 11 h, respectively. The Fischer rats showed a longer retention of PFC particles in the circulating blood than the Wistar rats.

Distribution of PFC

After injection with 20 ml/kg of PFC-E, the PFC contents in main organs were determined. Figure 3a, b) shows the changes in PFC contents of the Wistar rats bearing Walker 256 carcinosarcoma cells and the normal Wistar rats, respectively. Figure 4a, b) shows the PFC distribution in the Fischer rats bearing 9L glioma and the normal Fischer rats, respectively.

Wistar/Walker (Fig. 3a): The most PFC-abundant organ was spleen, in which the maximum PFC content reached to about 100 mg/g 24 h after injection, and then decreased gradually. In the liver, about 20 mg/g of PFC were

detected 24 h later. As the weight of liver was ten times as heavy as spleen, the amounts of PFC accumulated in the liver corresponded to approximately 30% of the injected dose. The PFC contents in the bone marrow were as much as liver. The PFC contents in the lung remained much lower levels comparing with the spleen and liver, and reached to the maximum one hour after injection. Whereas, the adipose tissues showed the opposite pattern in the PFC distribution against the other tissues. The PFC contents gradually increased during the observation period (until at least one week). The PFC particles were abundantly distributed in the implanted Walker tumor. The contents in the tumor were as much as those in the liver. One day after injection, they reached to the maximum level (about 20 mg/g), which corresponded to approximately 100 times as much as in the normal muscle, and then decreased. Although the growth of the Walker tumor was very rapid, the total amounts of PFC in the tumor did not decrease during the first week after injection.

Figure 3b) shows the results of the normal

Fig. 4. PFC Contents in Main Organs after Injection of PFC-E in 9L Glioma-Bearing Rats (a) and Normal Rats (b)
 —▼—, liver; —■—, spleen; —○—, lung; —□—, bone marrow; —△—, adipose tissue; —●—, tumor;
 ---●---, blood; ---×---, muscle.

Wistar rats having no tumor. The changes in PFC contents in each of the tissues almost coincided with those of the tumor-bearing rats. There were abundant PFC which was 5% of the injected dose at 24 h in the Walker 256 carcinosarcoma, but the accumulation of PFC in main organs were not influenced at all by the PFC accumulation in the tumor.

In Figs. 4a and b), the experimental results obtained using the Fischer rats are given. The obvious difference was the amount of PFC taken up into the 9L glioma as compared with the Walker carcinosarcoma. The maximum content in the 9L glioma was 2.5 mg/g after 24 h, which was only one tenth that of Walker 256 carcinosarcoma cells.

The incorporation of PFC into main organs of the Fischer rats resembled that of the Wistar rats with few exceptions. The bone marrow of the Fischer rats held 30 to 40 mg/g of the PFC level, which was about two times as much as that of the Wistar rats. Any significant differences in PFC contents in the liver, spleen, lung and kidney were not found between the 9L glioma-bearing rats and the normal rats.

Discussion

We had two purposes in the PFC distribution studies using tumor-bearing rats. The first was to compare the behavior of PFC-E in the normal rats with the tumor-bearing rats. The second was to find some basis for the tumor-dependent

efficacy of PFC in the 9L glioma over that in the Walker tumor.

The distribution of PFC in the main organs and tissues of the tumor-bearing rats were compared with the normal rats, after the rats were injected with PFC-E. The PFC retention of the normal animals has already been reported,¹⁵⁾ but the PFC distribution in the tumor-bearing animals has not been reported. The PFC accumulation into the tumors, either Walker tumor or 9L glioma, showed no clear effects on the distribution of PFC into the main organs and the PFC retention in the circulating blood. As a result, the innoculated tumors did not substantially affect the behavior of the PFC emulsion injected intravenously.

A slight but noteworthy difference was observed in the elimination rate of PFC emulsion from the circulating blood between the Wistar and the Fischer rats. In the Fischer strain rats PFC particles were retained longer than in the Wistar rats. The PFC particles are mainly taken up into the reticulo-endothelial system (RES) organs, such as the liver and spleen, as reported elsewhere.^{14,15)} As shown in Table II, the five main organs of the Fischer rats were smaller than those of the Wistar rats. The average weights of the liver and the spleen of the Fischer rats were 83% and 60% of those of the Wistar rats, respectively, although the average body weights of both the rat strains were the same. With the PFC concentration in the spleen and the liver, almost the same amounts were detected in both the strains

TABLE II. Organ Weights and PFC Contents in Main Organs after Injection of PFC-E

Organs	Walker 256 - Wistar rats			9L - Fischer rats		
	Organ weight (g)	PFC contents % of dose		Organ weight (g)	PFC contents % of dose	
		24 h	48 h		24 h	48 h
Liver	11.3 ± 0.8	25.1 ± 3.5	27.4 ± 2.0	9.4 ± 1.0 ^{a)}	20.3 ± 2.2 ^{a)}	21.9 ± 2.0 ^{a)}
Spleen	1.0 ± 0.1	14.5 ± 0.8	15.6 ± 3.5	0.6 ± 0.0 ^{a)}	8.6 ± 0.8 ^{a)}	8.8 ± 0.5 ^{a)}
Lung	1.2 ± 0.1	0.6 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.5 ± 0.1	0.4 ± 0.0
Kidney	2.0 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	1.8 ± 0.2	0.4 ± 0.1	0.2 ± 0.0
Hewart	0.8 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Tumor	4.8 ± 0.9	5.1 ± 1.9	4.3 ± 0.8	1.5 ± 0.2 ^{a)}	0.2 ± 0.1 ^{a)}	0.1 ± 0.1 ^{a)}
(Body)	234 ± 4			235 ± 6		

Results are expressed as the mean ± S.D. ^{a)}*p* < 0.05, compared with the value of Wistar rats.

(Figs. 3 and 4), but some differences in the PFC contents (% of dose) were found corresponding to the differences in the organ weights (Table II). These results suggest that maximum limits may exist in the PFC to be taken up into the liver and spleen, and that the elimination rate of PFC particles may be influenced by the RES organs' size.

The relatively great amounts of PFC were taken up into the Walker 256 carcinosarcoma implanted into the rat femoral muscle. The maximum concentration of PFC reached 20 mg/g of the tissue weight, corresponding to about 5% of the injected dose in the total tumor mass. On the other hand, the PFC contents in the 9L glioma were only one tenth as much as those of Walker tumor. The efficacy study with 9L glioma was done on the systematical basis. Korosue showed that a PFC emulsion with the oxygen inspiration had a synergistic effect on the radiation therapy for 9L rat glioma cells implanted intracerebrally.¹¹⁾ The data obtained in our study, however, showed that the PFC accumulation in 9L glioma was very low and the PFC accumulation in Walker tumor was ten times as much as that of 9L glioma. It has been thought that the Walker tumor was a tumor in which enhancing effects of the PFC-E could not be expected in the chemotherapy.¹²⁾ It may be that the radiosensitizing effects of PFC-E is not related to the PFC accumulation in tumor. We thus believe that the role of the PFC-E in radiotherapy is not to be taken into the tumor but is to pass the hypoxic tumor cells to transport oxygen.

The difference in the PFC accumulation between Walker 256 carcinosarcoma and 9L glioma may be explained by the difference in the growth rate between the two tumors. When the Walker tumor grows in size rapidly (Table II), the permeability of the vessel near the tumor will increase, and the PFC particles may leak out from the vessel and deposit in the tumor mass.^{16,17)} Otherwise, lots of macrophages will migrate around the tumor with the tumor growth and phagocytize the PFC particles as a foreign matter. The latter phenomenon was observed using CT scanning with similar PFC emulsions having a bromine atom¹⁸⁾: The VX 2 carcinoma implanted into the rabbit liver showed the radio-dense tumor-rim after the injection of a per-

fluorooctylbromide emulsion.¹⁹⁾

From these studies, we concluded that PFC blood levels and contents in the main organs do not alter between the normal and tumor-bearing rats. These findings also support the view that the enhancing effect of PFC-E is not related to the PFC accumulation in tumors.

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