Pathochemical Toxicity of Perfluorocarbon-5070, a Liquid Test Performance Fluid Previously Used in Dialyzer Manufacturing, Confirmed in Animal Experiment

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In the light of clustered deaths in late 2001 associated with hemodialysis (HD), this article analyzes the pathochemical toxicity of the perfluorocarbon-5070 (PF-5070), a liquid used as test performance fluid for detecting capillary leaks during dialyzer manufacturing. Residual PF-5070 in some Athane dialyzers of the involved brands was infused in the injured patients during hemodialysis. The clinical presentation was in contrast with other previously described severe reactions to HD. Foam material was discovered in the right ventricle and caval vein of the patients who underwent postmortem examination. Deaths were attributed to gas embolism without the external causes identified. To explore the pathochemical toxicity of the inert liquid PF-5070, an animal model was developed. In a rabbit model, single slug intravenous injections as bolus of increasing doses of PF-5070 were performed. In a first set of experiments, three groups of three rabbits were administered increasing doses of PF-5070 at 4, 40, or 160 \( \mu \text{L/kg} \). After intravenous injection, the animals were observed for clinical signs of adverse effects and underwent autopsy after death. Doses were normalized to animal body weight to allow comparison with supposed patient exposure. Five of nine rabbits died soon after PF-5070 dosing: One rabbit died within 4 h in the 4 \( \mu \text{L/kg} \) group, one rabbit died within 30 min in the 40 \( \mu \text{L/kg} \) group, and three rabbits died within 30 min in the 160 \( \mu \text{L/kg} \) group. In a second set of experiments, six rabbits were injected with a lethal dose of PF-5070 to analyze clinical symptoms and pathophysiology. All rabbits died on the day of dosing and displayed neurologic disorders (paralysis, nystagmus, rigidity, convulsions), then breathing abnormalities (rapid breathing, salivation, dark mucous membrane), and fatal collapse. Autopsy of rabbits showed evidence of gas retention in the lung tissue and gas bubbles in the right cardiac cavities. Histologic findings included alveolar hemorrhage with pulmonary edema, cerebellum, and cortex patchy areas of infarction. Single-dose intravenous administration of PF-5070 reproduced in a rabbit model the pathophysiologic effects observed in the hemodialysis patients. Severity of the symptoms observed in the animals was dose-dependent. Clinical and pathologic findings can be explained by the capacity of perfluorocarbon to emulsify blood at body temperature, to increase partial pressure in the pulmonary capillary bed, and to form bubbles in the pulmonary capillary circulation, thus blocking lung and visceral perfusion. Such experimental findings indicate the toxicity of PF-5070 administered intravenously and make the pathochemical toxicity link with the hemodialysis-related deaths caused by the presence of residues of PF-5070 in the Athane dialyzers. We conclude, in light of this outbreak and the subsequent investigations, that liquid PF-5070 is a highly toxic compound when administered intravenously because of its emulsifying properties. The use of PF-5070 or any liquid fluorocarbon compounds in medical devices with blood contact and particularly in the dialyzer manufacturing should be considered with caution.


The deaths in 2001 associated with the use of Athane series dialyzers has drawn the attention of the nephrology community to a new hazard of maintenance hemodialysis (1–4). The mode of presentation of these tragic events differs significantly from what has been described in the past. The common features of this new dialysis-related pathologic event entitled the “perfluorocarbon syndrome” (5) were that it occurred as clustered events within a discrete period of time in different geographic locations (Spain, Croatia, the United States) with different clinical practices; it was not comparable to the “first-use syndrome” nor did resemble a hypersensitive reaction; it occurred during or soon after the hemodialysis session; it appeared to be lethal in all cases despite conventional and intense resuscitation maneuvers; and foam was identified in blood mainly of the right ventricle in autopsied Croatian...
patients, leading to speculation that death was caused by gas embolism. During the investigation, two important observations were made leading to the discovery of the causal agent: Only two particular brands of dialyzers were used in all patients; residues of liquid perfluorocarbon (PF-5070) compound, a liquid test performance fluid, was identified in some recalled dialyzers.

To elucidate the pathophysiologic role of the PF-5070 in this tragedy, it was decided to develop an experimental animal study based on a single intravenous injection of commercially available PF-5070. In this article, we report the experimental data achieved during the course of this study confirming the toxicity and the original pathochemical role of these volatile compounds.

Materials and Methods

**PF-5070**

PF-5070 is a volatile perfluorocarbon (perfluorohexane, C$_7$F$_{16}$) presenting as a liquid form with a density of 1.7 g/ml and a relatively high vapor pressure of 79 mmHg at 20°C, and is produced by the 3M Company. PF-5070 has been used in the dialyzer industry to detect dialyzer fiber leaks after freon usage was prohibited to protect the environment (6,7). This test performance liquid was then removed from dialyzers through an air pressure system that forces air through the environment (6,7). This test performance liquid was then removed from dialyzers through an air pressure system that forces air through the environment, thus totally eliminating the PF-5070.

**Toxic Role of PF-5070 in an Animal Model**

To explore the toxicity of the PF-5070, an animal study was designed based on single bolus intravenous injection. Rabbits were selected as the test animal for convenience because of the low weight and the high sensitivity of these animals to the effects of PF-5070 injected intravenously. In all cases, animal experiments scrupulously respect the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Two series of animal experiments were performed under general anesthesia. The first set of experiments was conducted at Baxter R&D, Chicago, IL, and was performed with dose increments of PF-5070 in three groups of three rabbits weighing 2590 ± 250 kg to determine the threshold toxicity of this compound. The second set of experiments was conducted at Renal Research Laboratory of Cordoba, Spain, and was performed in rabbits weighing 2540 ± 210 kg, using a single, massive, intravenous injection of PF-5070 to analyze the pathochemical toxicity in terms of clinical side effects and tissue damage. PF-5070 dosing was estimated according to the amount of PF-5070 recovered in certain recalled dialyzers ranging from 350 mg to 6400 mg (Baxter R&D communication, August 2002).

In the first set of experiments, groups of rabbits were administered a single intravenous injection of PF-5070 at 4, 40, or 160 µl/kg. After dosing, the animals were observed for clinical symptoms or adverse effects.

Doses of PF-5070 used were normalized to animal body weight to allow comparison with patient exposure. Given a PF-5070 density of approximately 1.7 g/ml, the volume doses of 4, 40, and 160 µl/kg correspond to doses of 6.8, 68, and 272 mg/kg. The lowest dose of PF-5070 administered to rabbits corresponds to a 60-kg patient exposed to approximately 408 mg PF-5070. The highest dose tested (160 µl/kg) corresponds to a 60-kg patient exposed to approximately 16,320 mg PF-5070.

In the 4-µl/kg dose group, one of three animals died within 4 h after injection. That animal displayed clinical signs of lateral decumbency, paraparesis, rigidity, unequal pupils, loss of righting reflex, facial paralysis, and reduced toe pinch reflex.

In the 40-µl/kg dose group, one of three animals died within 30 min after the injection. This animal displayed rear leg weakness, unequal pupils, open-mouth breathing, horizontal nystagmus, dark mucosal membranes, and a slow and irregular heart rate.

In the 160-µl/kg dose group, all three animals died within 30 min after the injection. Clinical signs included rigidity, convulsions, dark mucosal membranes, rapid breathing, salivation, paralysis, and unequal pupils. It is of note that all rabbits died on the day of intravenously injection.

In the second set of experiments, six rabbits received a single, massive, intravenous injection of PF-5070 equivalent to 160 µl/kg. After injection, the animals were observed for clinical symptoms of adverse effects. All rabbits presented soon after with rapid breathing, dark mucosal membranes, convulsions, paralysis, and rigidity. All died within 30 min of injection and an autopsy was performed.

Necropsy data showed evidence of gas retention in the inferior vena cava and in the lung tissue, as indicated by partial or incomplete collapse of the lung after the thoracic cavity was opened. In the rabbits that received the massive dose of PF-5070 (160 µl/kg), gas bubbles were noted in the right atrium and in the right ventricle (Figure 1). The rabbits given the low or intermediate dose (4 or 40 µl/kg) had mostly generalized malacia of the cerebellum and in the brain.

Histologic changes in lung sections included presence of air in the lung capillaries, primarily involving alveolar hemorrhage and/or edema, infarction (Figure 2), and inflammation of the pulmonary vasculature (Figure 3), and bubbles in the coronary artery (Figure 4). Pulmonary infaracts were detected in all rabbits given the high dose (160 µl/kg) and in some rabbits given the intermediate dose (40 µl/kg). Alveolar hemorrhage, edema, and inflammatory changes were detected in virtually all rabbits.

Thus, the histologic findings associated with the intravenous administration of PF-5070 involved the vascular system and were accompanied by ischemic organ damage secondary to vascular injury or obstruction. Microscopically, gas bubbles were recognizable as clear spaces (vacuolar spaces) in the intravascular compartment silhouetted by blood, which filled the remaining available space (Figures 5 and 6).

**Figure 1.** Heart of a rabbit postautopsy after intravenous injection of PF-5070 at a bolus dose of 160 µl/kg showing the presence of macroscopic air bubbles in the right atrium, confirming air embolism.
All rabbits had intravascular vacuolar spaces in the lung and some showed the same type of lesions in the liver, kidneys, and spleen. The rabbits given the high dose had microscopic hemorrhages in the central nervous system. Some rabbits also showed an extensive area of necrosis involving the cerebellum and spongy degeneration in the cerebral neocortex. The necrosis was sharply demarcated from adjacent viable tissue, a characteristic morphologic sign of an ischemic infarct.

Discussion
The pathochemical role of PF-5070 administered intravenously has been established by this animal study. Given the similarity of clinical symptoms and histopathologic findings observed in dialysis patients and experimental animals, it may be confirmed that PF-5070 was the agent responsible for the multi-organ damage. Respiratory failure and cardiac arrest are easily explained by the formation of gas bubbles (foam aspect) blocking the microcirculation of the lung and right heart and being responsible for disseminated patchy areas of ischemia and infarction in the affected organs (lungs, heart, brain cortex, and cerebellum).

PF-5070 was used by some dialyzer manufacturing factories, being considered an inert, nontoxic, and nonirritant compound. This particular compound had not been tested for direct intravenous toxicity. The toxicity data for perfluorocarbon compounds found in scientific literature were based mainly on oral, topical, pulmonary, and intraperitoneal routes of administration. Moreover, the fact that perfluorocarbon-derived com-

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**Figure 2.** Mottled lung confirming the presence of multiple areas of infarction in a rabbit postautopsy after intravenous PF-5070 injection at a bolus dose of 160 µl/kg.

**Figure 3.** Heart of a rabbit postautopsy after intravenous injection of PF-5070 at a bolus dose of 160 µl/kg, showing the presence of macroscopic air bubbles in the right coronary artery.

**Figure 4.** Microscopic section of a rabbit’s lung postautopsy after intravenous injection of PF-5070 at a bolus dose of 160 µl/kg. Note the presence of foam material in the capillary lung. It is interesting to note that microbubbles are passing through the capillary wall in some capillaries.

**Figure 5.** Enlarged microscopic picture of a small pulmonary artery lung illustrating the passage through the capillary wall of microbubbles.
pounds have been tested in animals as oxygen-carrying blood substitutes with some success may have contributed to give a false safety to these compounds (8,9). Note that in previous animal studies the intravenous injection of fluorocarbon (FC) compounds was associated with severe, clinical, and systemic adverse effects in dogs (10–12). In addition, toxicity of FC compounds was related to the chemical characteristics of the FC compound itself (FX-80, FC-43). Interestingly, FC-43, sharing physical properties similar to PF-5070, was responsible for severe systemic toxic effects in animals (13). Histopathologic findings were similar to those observed in our experiment showing extensive pulmonary lesions (edema, hemorrhages, infarction) and disseminated patchy lesions of kidney (necrosis), liver, and spleen. We conclude that regardless of its inert nature, in vivo slug injection of FC was rather toxic. At the time of dialysis events, FC compound hazards were not recognized by dialyzer manufacturers. Residues of FC compounds in the dialyzers were not detected in the battery of toxic tests required by notified bodies and/or International Organization for Standardization (ISO) testing procedures before releasing new dialyzers on the market.

From data collected in this study, one can hypothesize the sequence of events leading to this particularly severe and refractory cardiopulmonary failure. PF-5070 released from implicated dialyzers was infused intravenously quite quickly and at different amounts during the hemodialysis session. The PF-5070 quickly reached the pulmonary circulation and accumulated in the pulmonary capillary circulation because of the impermeability of the capillary membrane. Complete evaporation of PF-5070 at body temperature must have increased the intravascular total gas volumes. PF-5070 failing to cross the pulmonary alveolar capillary membrane accumulated into the pulmonary. Accordingly, PF-5070 increased the partial pressure in the pulmonary capillary blood and not the alveolar air, contributing to an increase in the capillary to alveolar partial pressure gradient. As a consequence, bubbles of CO₂, O₂, N₂, PF-5070, and water were formed progressively in the pulmonary capillary circulation, exceeding the expansion capacity of the capillary bed, passing to some extent through the lung to the left side of the circulation but mainly back-filling to the pulmonary artery and right ventricle cavities. By blocking the microcirculation, air bubbles might subsequently induce severe organ dysfunction and ischemic damage leading to infarction (lungs, heart, brain).

Production of air bubbles into the blood of the animal follows the basics of physics and fluid dynamics. According to the ideal law, 1 mol of any gas occupies a volume of 22.4 L at standard temperature and pressure. Given that PF-5070 has a molecular weight of 388 and a density of a 1.7 g/ml, complete evaporation of 1 µl of liquid PF-5070 would yield approximately 98 µl of PF-5070 gas. Theoretically, complete evaporation of liquid PF-5070 administered intravenously to rabbits weighting 2.5 kg and having a normal body temperature of 37.5°C could have yielded intravascular total gas volumes in excess of 1 ml in rabbits given 4 µl/kg, 10 ml in rabbits given 100 µl/kg, and 40 ml in rabbits given 160 µl/kg. Approximating amount of PF-5070 infused accidentally to dialysis patients to be in the range of 350 to 6400 mg, one can estimate the volume of bubbles produced by the total emulsification of perfluorocarbon was in the range of 10 to 50 ml for a 60-kg patient.

At this stage of the discussion, one can establish the pathochemical toxicity of intravenously administered PF-5070. It is important to remember that under certain conditions of body temperature and BP, an inert liquid FC compound might become volatile and promote the acute formation of gas bubbles similar to air embolism. Severe and unforeseeable hazards associated with the use of PF-5070 and some perfluorocarbon compounds should ban their use in dialyzer testing and more generally in medical devices that might have some blood contact.

**Conclusion**

This animal study has established the pathochemical role of PF-5070 when administered intravenously because of its emulsifying power at pressure and body temperature. The similarity observed in dialysis patients and experimental animals suggests that PF-5070 was the causal agent of bubble formation in blood mimicking gas embolism and was responsible for multiorgan damage. For safety purposes, the use of PF-5070 and perfluorocarbon compounds should be avoided in medical devices with blood contact and particularly in dialyzer manufacturing.

**Acknowledgments**

The authors thank the “Fundacion Nefrologica of Spain” and the “Baxter Research Center” (Dr. Jose Divino, Baxter Europe, Brussels-Belgium) for supporting this experimental study, and the Center for Artificial Organ Development Department of Surgery (Prof. Y. Nosé), Houston, TX, for its scientific advice.
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