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Prolongation of the Intestinal Viability Using Oxygenated Perfluorocarbon in an Experimental Model of Acute Intestinal Ischemia

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Objectives. *Liquid perfluorocarbons (PFCs) are well known for their capability to carry respiratory gases. The aim of this study was to evaluate the effectiveness of oxygenated F-Decalin on the intestinal viability, in an experimental model of acute intestinal ischemia.*
Material and Methods. *Thirty-six rabbits were subjected to 8 h intestinal ischemia by ligation of the superior mesenteric artery (subgroups 1), the mesenteric vein (subgroups 2) or both vessels (subgroups 3). The animals were divided into three groups: (a) Control (ischemia alone), (b) PFC-O_2 (ischemia plus infusion of oxygenated F-Decalin) and (c) PFC (ischemia plus infusion of not-oxygenated F-Decalin). Intestinal biopsies from four different sites and blood samples for serum enzymes measurements were taken at 2, 4, 6 and 8 h. All tissue sections were examined blindly under light microscope. Sections from the specimens were taken at 4 and 8 h, and examined blindly under the electron microscope. Statistical analysis was performed by non-parametric Kruskal Wallis test.*
Results. *Using light microscope, the observed intestinal damages to the sections from Control and PFC groups were severe at 4 h and destructive after 8 h. On the contrary, minimal injuries were observed in the biopsies from PFC-O_2 group at 4 and even after 8 h of ischemia. These findings were confirmed by the electron microscope study and correlated to the serum enzymes measurements.*
Conclusions. *These results suggest that intestinal viability could be prolonged after acute ischemia using oxygenated perfluorocarbons and this could be a promising pretreatment modality for a variety of mesenteric ischemic forms.*

Keywords: Perfluorocarbons; F-Decalin; Acute intestinal ischemia.

Introduction

Acute intestinal ischemia is a devastating event. The associated morbidity and mortality from the regional or systemic complications[1–3] are approximately 60–70%.[4,5] and the delay in diagnosis and treatment may be fatal for the patient.[4,6,7] Surgical treatment is often delayed due to hemodynamic instability or poor general condition of the patients (i.e. recent myocardial infarction, multiple organs failure). Furthermore, nearly half of the cases of acute intestinal ischemia are caused by non-obstructive causes[8] and develop in already hospitalized, critically ill, patients.[9] The aim of this study was to evaluate the effectiveness of intraluminal administration of oxygenated perfluorocarbons on the intestinal viability during an acute intestinal ischemic episode.

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Material and Methods

Subjects

A total of 36 rabbits (White New Zealand, males, 6 months old, mean weight of 3096 ± 203 g) were used for this study. The experimental protocol was approved by the Scientific and Ethical Committee of our Institution. The animals were divided in three groups (Control, PFC-O_2 and PFC). Control group (n = 12) included intestinal ischemia alone. In PFC-O_2

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group ($n=12$) ischemia was combined with intraluminal administration of oxygenated F-Decalin while in PFC group ($n=12$) ischemia was combined with intraluminal administration of not-oxygenated F-Decalin. The animals of each group were subjected to 8 h of intestinal ischemia by ligation of the superior mesenteric artery (subgroups 1), the mesenteric vein (subgroups 2) or both mesenteric vessels (subgroups 3).

Liquid perfluorocarbons (PFCs) are biologically inert compounds well known[10] for their high capability to carry respiratory gases to (and from) the tissues and have already been used in experimental studies of ischemia-reperfusion injury[11–13]. In this study were used Perfluorodecalin (F-Decalin, Fluoron GmbH, Neu-Ulm) which is a pure bicyclical fluorocarbon (formula $C_{10}F_{18}$ and m.w. 462). The F-Decalin was bubbled with 100% oxygen as previously described by Sloviter and Muckerji.[10,11,14]

Experimental design

Animal model

The total duration of the experimental ischemia was 8 h. Animals were sedated with i.m. ketamine hydrochloride (20 mg/kg*) plus xylazine hydrochloride (2 mg/kg*). The femoral vein was dissected through a short longitudinal incision in the right thigh and anesthesia was achieved and maintained using i.v. Sodium Pentobarbital (2 mg/kg*/h). A midline laparotomy was performed and the mesenteric vessels were ligated (5-0 silk). Making a short longitudinal incision in the left thigh, the common femoral artery was dissected for monitoring the vital signs. During the experiments, animals received i.v. 0.9% NaCl solution and the mean infusion rate was approximately 60 ml/h. A small prepyloric gastrotomy was performed and a thin catheter (20 cm length) was placed to the animal's duodenum. The tip of catheters reached the duodenal end and these were used for the administration of PFC-O_2 or PFC. The fluorocarbon flow was maintained at a constant rate of 15 ml/h and the total amount was approximately 120 ml. Antimesenteric wedge intestinal biopsies were taken at 2, 4, 6 and 8 h after the onset of ischemia, with care for preservation of the intestinal lumen continuity. In order to examine the total length of the animal's small bowel, four biopsies were taken at each time interval mentioned above, at 20–40–60 and 80 cm from the duodenal end. Venous blood samples were taken at the same time intervals for measurements of LDH, CPK, SGOT, SGPT, inorganic Phosphorous and routine hematological tests. After the ligation of mesenteric vessels, the midline laparotomies were closed using a running 3/0 suture (Prolene, Ethicon) and the same procedure was followed every 2 h when the abdomen was reopened for taking the biopsies.

Light microscope study

The intestinal tissue samples were prepared in BOUIN solution for 2 h and consecutively in 10% formaldehyde. The sections were stained with hematoxylin–eosin and were studied under the light microscope. A total of 576 observations were made, 192 for each group and 64 for each subgroup. The sections were examined blindly and graded in five grades of ischemic injury according the following criteria[13] (Table 1).

Electron microscope study

The observed sections were taken from all the animals at 4 and 8 h after the onset of ischemia and from the distances of 40 and 80 cm from the duodenal end. A total of 48 observations were made for each group on a JEOL 2000 FX II TEM electron microscope.

Serum measurements

The centrifuged serum were used for the measurements of LDH, CPK, SGOT and SGPT levels on a Hitachi 4020 Boehringer Mannheim analyzer using ultraviolet photometry at 340 nm and the results are expressed in i.u./l. The measurements of inorganic Phosphorous were made on an Integra 400 Roche analyzer using also ultraviolet photometry at 340 nm and the results are expressed in mg/dl.

Statistical assessments

Statistical analysis of continuous data (serum measurements) was performed between equal subgroups by non-parametric Kruskal Wallis test due to small number of animals of each subgroup ($n=4$). The same non-parametric test was used for the analysis of categorical data (grade of intestinal ischemia). Analysis was performed for all time intervals: (a) between the four different sites of biopsies of each subgroup, (b) between the equal sections of subgroups of the same group and (c) between the equal subgroups of the three groups for the same distance and ischemic cause. Statistical significance was set at a level of $p<0.05$. Analysis was performed with the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA).

Table 1. Criteria for the histological grading system

Grade	Histological appearance	Characterized 'as…'
0	Normal	
1	Partial separation of apical cells, mild subepithelial edema	'reversible'
2	Epithelial cell slough from the tips of villi, preservation of lamina propria	'possible reversible'
3	Extended partial mucosal necrosis, degradation of lamina propria	'possible non-reversible'
4	Complete mucosal necrosis, transmural necrosis	'non-reversible'

Results

Light microscope study

Intestinal biopsies of animals of the same subgroup revealed that ischemic injuries were similar at the same time interval between the four different sites of biopsies and with no statistical significance. The ischemic damage for the same time interval (2, 4, 6, 8 h), was also similar for the three subgroups (1, 2, 3) of each group (Control, PFC-O_2, PFC).

The biopsies of Control group demonstrated severe ischemic damages after 2 h of ischemia. At 4 h extended partial mucosal necrosis and degradation of the lamina propria was observed (Fig. 1a). Between the sixth and eighth hours, complete necrosis and homogenization of the mucosa and submucosa layers was observed (Fig. 2a). Similar damages were demonstrated in the equal sections from PFC (not-oxygenated F-Decalin) group. Statistical analysis, for the same time interval, distance from the duodenum end and ischemic cause, between Control and PFC group showed no statistically significant difference.

On the contrary, the observations of the PFC-O_2 (oxygenated F-Decalin) group showed minimal histological alterations. In the segments that were taken at fourth hours of ischemia only partial separation of apical cells and mild subepithelial edema was observed (Fig. 1b). At 8 h partial epithelial cell sloughing from the tips of villi were observed while the lamina propria and the Lieberkuhn's cache were preserved. No further injuries were demonstrated in the submucosa or to the muscle layers (Fig. 2b). The $PFCO_2$ subgroups showed significantly less ischemic alterations compared to the respective subgroups of Control and PFC groups at all time intervals.

Electron microscope study

These observations were used for the accurate definition of the ischemic injuries on the cellular and intracellular level. As has been already shown by the light microscopy study, the observed sections from the Control and PFC groups had ischemic damages of similar severity. At 4 h the sections from these groups showed complete loss of the epithelium, partial destruction of the basal lamina and capillaries congestion with severe edema and degradation of the lamina propria (Fig. 3). At 8 h massive necrosis and homogenization of the mucosa and submucosa tissues were observed with residual evidence of cells and organelles lyses (Fig. 4a). On the other hand, the sections from the PFC-O_2 group showed minimal injuries. At 4 h, the appearances of the mucosa were comparable to the normal and mucosal integrity was

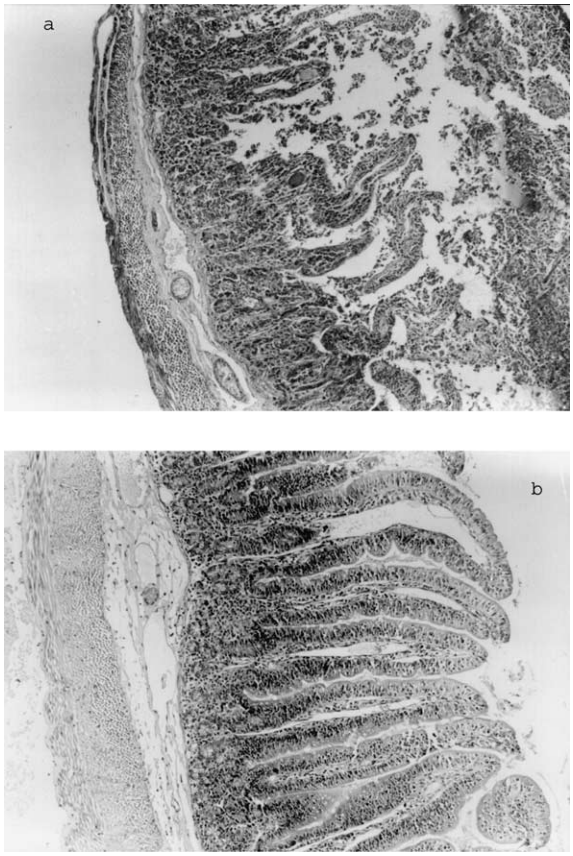

Fig. 1. (X25) Histological appearance at 4 h after ligation of both mesenteric vessels. (a) Control group, (b) PFC-O_2 treated group.

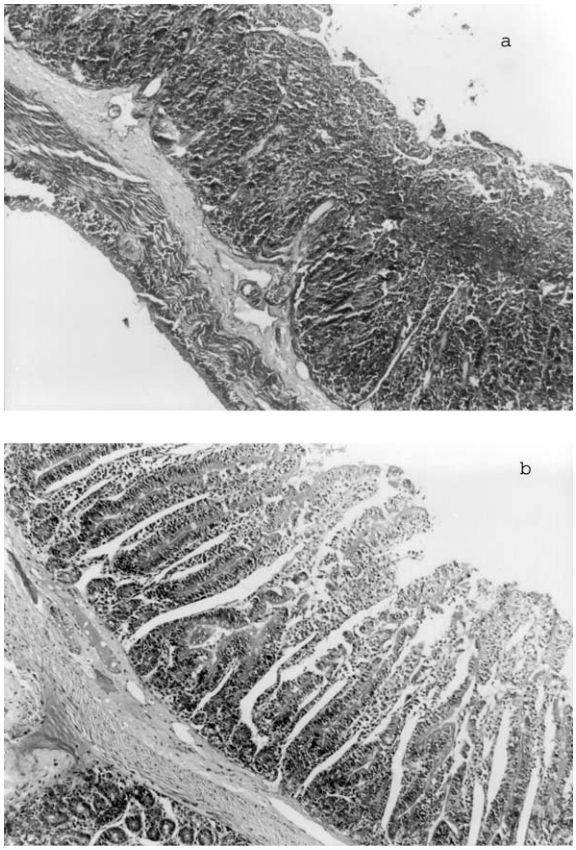

Fig. 2. (X25) Histological appearance at 8 h after ligation of both mesenteric vessels. (a) Control group, (b) PFC-O_2 treated group.

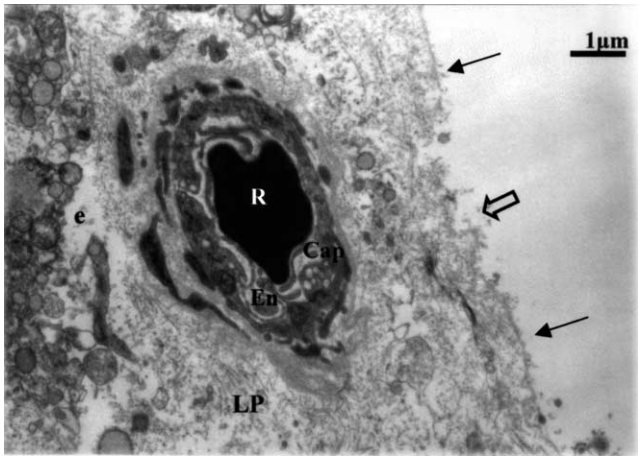

Fig. 3. Control group, electron microscopic appearance at 4 h after ligation of both mesenteric vessels. (Thin arrows show complete loss of the epithelium, thick arrow shows partial destruction of the basal lamina, Cap: capillary vessel, En: endothelial cell, R: red blood cell, LP: Lamina Propria, e: edema.)

maintained even after 8 h of ischemia. The cellular architecture, the desmosome junctions between the intestinal epithelial cells and the intracellular organelles remained intact (Fig. 4b).

Serum measurements

The Lactic Deydrogenase (LDH) levels of the animals of Control and PFC groups increased at 2 h of ischemia and progressively to the eighth hour exceeded the levels of 2000 i.u./l. There was no significant difference at the same time interval, between subgroups of the same group and between the equal subgroups of Control and PFC groups. On the other hand, the increase of LDH on the PFC-O_2 group measurements was retarded and reached the maximum of 500 i.u./l at 8 h. LDH measurements compared between equal subgroups of PFCO_2, Control and PFC groups showed statistical significance at all the time intervals (Fig. 5). The results were similar for SGOT and SGPT but the differences were statistically significant only at 6 and 8 h after the onset of ischemia. Differences mentioned above were magnified in the measurements of Creatine Phosphate Kinase levels. CPK measurements of Control and PFC groups reached at 4 and 8 h the levels of 4000 and 7000 i.u./l respectively, while in the PFC-O_2 group levels did not exceed 1500 and 2000 i.u./l. Comparing CPK measurements between equal subgroups at all the time intervals we observed significant statistical differences.

Discussion

Attempts to prolong intestinal viability during an acute intestinal ischemic episode has already been reported by Shute in 1976.[15] Previous studies have shown that the intraluminal administration of oxygenated saline or gaseous oxygen protects intestinal mucosa and decreases the mortality rate in experimental animal models.[16,17] The mechanism of oxygen delivery of these methods makes them not feasible in clinical practice because of unwanted distension. The high gas solubility and the biological inertness of the perfluorocarbons are advantageous properties that make them attractive for many medical applications[18–21] and especially in intestinal ischemia.[13,22] The non-polar gases solubility is related to the molecular volume of the gas, which occupies PFC's intermolecular spaces and depends on partial gas pressure (i.e. pO_2) approximating to Henry's Law. In the intestinal lumen environment, with very low pO_2 levels, the PFC releases progressively oxygen and it

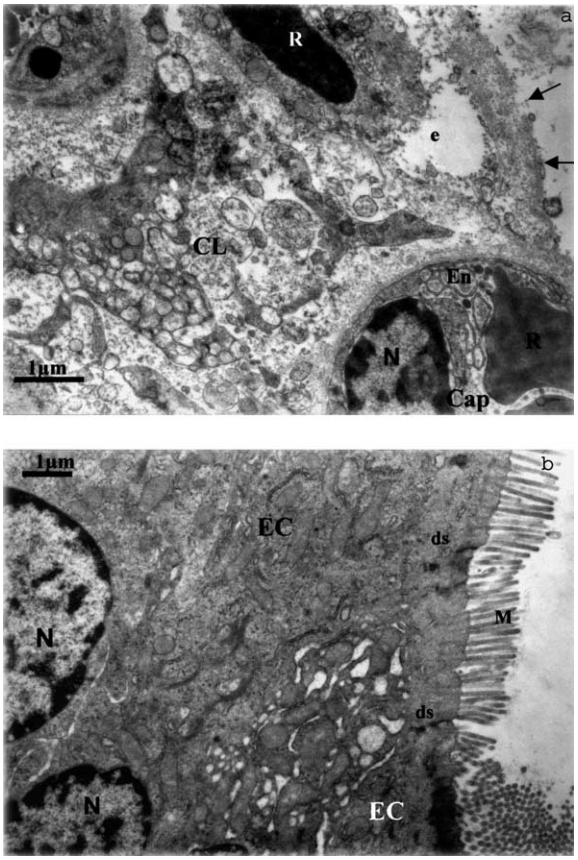

Fig. 4. Electron microscopic appearance at 8 h after ligation of both mesenteric vessels. (a) Control group (thin arrows show complete loss of the epithelium, Cap: capillary vessel, En: endothelial cell, R: red blood cell, CL: cells lyses, e: edema, N: nucleus). (b) PFC-O_2 treated group (M: microvilli, EC: epithelial cells, ds: desmosomes, N: nucleus).

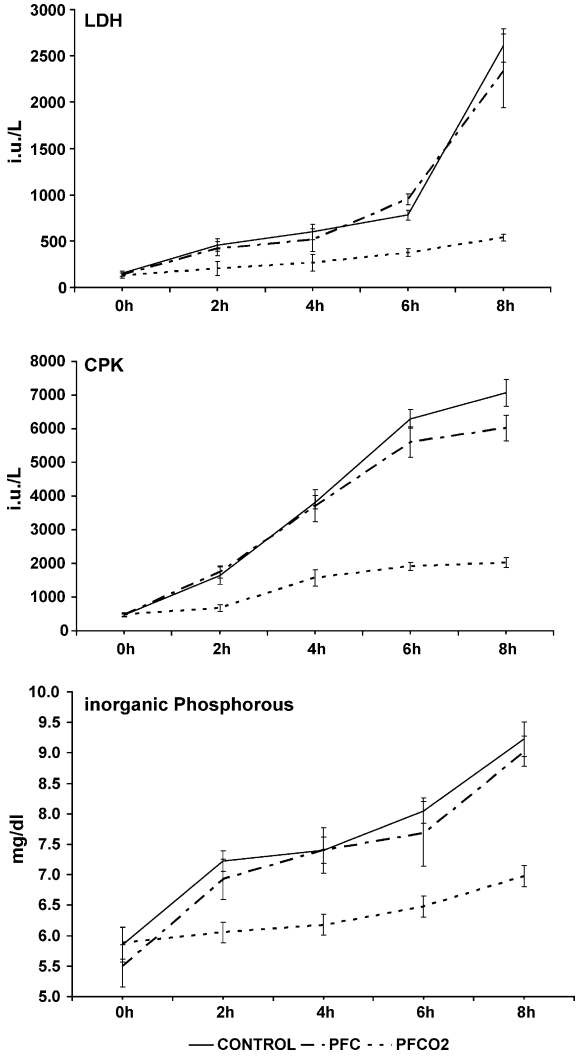

Fig. 5. Ligation of SMA (subgroups 1): mean LDH, CPK and inorganic Phosphorous.

passes through membranes by diffusion in the intracellular environment and organelles. Furthermore, the PFC's low viscosity and high density results in rapid passage through the intestinal tract. The small intestinal uptake cleared mainly by expired air.

Previous experimental studies have shown that the intraluminal administration of oxygenated PFC's protects the intestinal mucosa from ischemia or ischemia/reperfusion injuries.[13,17] However, the duration of ischemia to these studies was short, so that the offered time lag of prolonged intestinal viability was unrealistic for other pretreatment manipulations in clinical practice, especially for critically ill patients. The value of prolongation of the intestinal viability for several hours is obvious for patients who suffer from acute intestinal ischemic episode related to low flow state or with unstable hemodynamics.

Our results on light microscope showed that using intraluminal oxygenated fluorocarbon the intestinal histological alterations remains minimal even after 8 h of ischemia. The observations of the electron microscope study confirmed the above findings and showed clearly, that the intestinal mucosal cells remained intact with preserved structural and intracellular integrity.

In clinical practice the reconstruction of the mesenteric arteries carries a low mortality rate. The incidence of acute mesenteric infarction remains high and nearly half of the cases are due to non-obstructive causes.[8] Hospitalized, critically ill, patients are the main category of patients affected from this entity.[9] To these patients the severe ischemic damages may be fatal and reduces the 'golden period' for the confirmation of the diagnosis and the restoration of blood flow before bowel tissue loss is unavoidable.

Our study shows that continuous intraluminal intestinal flow of oxygenated perfluorocarbon for 8 h protects the rabbit's small bowel from the ischemic injuries. Also previous studies have shown that, in

addition to its high O_2 carrying capacity, PFCs have anti-inflammatory properties by decreasing neutrophills adhesion.[20,22]

Future directions of this study are to determine the effects of this technique in the prevention of the reperfusion injury by injecting this, non-cellular, oxygen delivery compound to the mesenteric vascular bed just before the restoration of arterial blood flow. It should be noted that passive diffusion of oxygen from the lumen, via the intracellular spaces, may not be sufficient to maintain the viability of submucosa and muscular layers of the thick human gut, in contrast to thin thin-walled rabbit small bowel.

The findings suggest that intraluminal intestinal administration of oxygenated perfluorocarbon may offer a promising pretreatment modality for a variety of mesenteric ischemic disorders especially in mesenteric insufficiency in the elderly and in ICU patients. What role, if any, the PFCs could play in future clinical practice is yet to be determined.

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