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Reversal of the Endocrine Toxicity of Commercially Produced Perfluorochemical Emulsion[1]

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ABSTRACT

Perfluorochemicals provide a biologically inert system for oxygen transport to tissue. The purpose of the present study was to determine if a simple clean-up procedure could reverse the endocrine toxicity of a commercially produced perfluorochemical emulsion, Oxypherol-E.T. The clean-up procedure consisted of a combined resin and dialysis treatment. The endocrine toxicity of the untreated and treated perfluorochemical emulsions was tested by determining their effect on testosterone secretion by rat testes perfused in vitro. Rat testes perfused with untreated Oxypherol-E.T. secreted low amounts of testosterone. However, the treated Oxypherol-E.T. was an effective and nontoxic oxygen carrier for testes perfused in vitro. The results are significant because they suggest that the endocrine toxicity of Oxypherol-E.T. is caused by toxic contaminants and not the perfluorochemicals. Additional experiments revealed that the fluoride ion may be the primary toxic contaminant of Oxypherol-E.T. The data support the efficacy of perfluorochemicals as oxygen carriers for rat testes perfused in vitro.

INTRODUCTION

Perfluorochemicals have proven to be effective oxygen carriers in numerous physiologic experiments (Riess and Le Blanc, 1982). A perfluorochemical emulsion designed for basic research has been developed and marketed by Green Cross Corporation, Osaka, Japan. The emulsion has been marketed as Fluosol-43, FC-43 Emulsion, and Oxypherol-E.T. In this report, the Green Cross emulsion will be referred to as Oxypherol-E.T.

Oxypherol-E.T. represents the *only* commercially available perfluorochemical emulsion. Advantages of a common source of perfluorochemical emulsions include more reliable comparisons of experimental results and the experimental use of perfluorochemicals without preliminary development of the required emulsification procedures. The major disadvantage of a commercially prepared perfluorochemical emulsion is that investigators assume that the product is effective under their experimental conditions. Subsequently, negative results are usually attributed to the perfluorochemicals.

Oxypherol-E.T. does not support the endocrine function of organs. Because the rat pancreas perfused with Oxypherol-E.T. did not secrete insulin, Ikeda and coworkers (1979) concluded that perfluorochemicals were the detrimental factor. Our earlier experiments (Chubb and Draper, 1985) established that Oxypherol-E.T. inhibited testosterone secretion by rat testes perfused in vitro. However, we determined that perfluorochemicals were not the toxic factor because our laboratory-produced perfluorochemical emulsion was not toxic (Chubb and Draper, 1985).

In this report, we demonstrate that the deleterious effects of Oxypherol-E.T. on endocrine function can be reversed by a simple clean-up procedure. The results are important because they suggest that previous failures of endocrinologic experiments incorporating Oxypherol-E.T. were due to toxic contaminants and not the perfluorochemicals. Furthermore, the experimental data indicate that the fluoride ion is the primary toxic contaminant of Oxypherol-E.T.

MATERIALS AND METHODS

Animals

Four male Sprague-Dawley rats were purchased

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from Charles River Laboratories (Wilmington, MA) and housed under constant conditions (23°C, 14 h light/24 h). Feed (Teklad 4%, Teklad Industries, Madison, WI) and water were available at all times. Animals (384–439 g body weight) were killed by cervical dislocation immediately before orchidectomy.

In Vitro Testis Perfusion

Testes were perfused in vitro according to a previously described method (Chubb and Desjardins, 1983; Chubb and Draper, 1985). Briefly, the capsular artery of an isolated testis was cannulated with a glass micropipette and perfused with one of the described perfusion media (see below). One testis of each animal was perfused with treated perfluorochemical emulsion; the contralateral testes were perfused with untreated perfluorochemical emulsion. All testes were perfused at a constant flow rate (20 ml/h) and maximally stimulated with luteinizing hormone (LH; NIADDK-oLH-24, 100 ng/ml). The testes weighed 1.62 ± 0.1 g. Perfusions were performed in a heated (32.5°C) and humidified environmental chamber. Testicular venous effluent was collected in chilled tubes for 30 min after a 1.5-h equilibration period.

Perfusion Media

Two categories of perfluorochemical media were tested for their efficacy as oxygen carriers for rat testes perfused in vitro. The components of each perfusion medium were identical (Table 1). Perfluorotributylamine (FC-43) and Pluronic F-68 were purchased as an emulsion (Oxypherol-E.T., Lot 039GI) from Alpha Therapeutic, Los Angeles, CA (a distributor for Green Cross Corporation) and used within 1 wk of shipment arrival.

The *only* difference between the two perfluorochemical media was the treatment of the Oxypherol-E.T. emulsion before it was mixed with the remainder of the perfusate. Fifty percent (200 ml) of the Oxypherol-E.T. emulsion was used as received. The remaining 200 ml of Oxypherol-E.T. from the same bottle was stirred with resin [6% (wt/vol) Amberlite MB-3; Sigma Chemical, St. Louis, MO] for 30 min at room temperature and filtered through a 0.8-μm filter (Type AA; Millipore, Bedford, MA). The filtrate was poured into 1.3 ft of dialysis tubing with a M_r cutoff of 6000–8000 (Spectra Por 1, cylinder diameter of 32.8 mm; Spectrum Medical, Los Angeles, CA) and dialyzed against 4 l of deionized water for 24 h at room temperature. The low osmotic pressure of the perfluorochemical emulsion (10 mOsmol) and the absence of empty space in the dialysis tubing account for the limited increase (∿8%) in sample volume. The clean-up procedure was developed for our laboratory-produced perfluorochemical emulsions (Chubb and Draper, 1985).

Physiochemical parameters of the two perfusion media were measured by previously described methods (Chubb and Draper, 1985) and were similar: osmolarity, 305 mOsmol; pH, 7.4; viscosity. 2.5 centipoise; optical density, 0.13.

Analysis of Steroidogenesis

Testosterone secretion was selected as the measurement of testicular steroidogenesis because testosterone is the primary steroid secreted by rat testes perfused in vitro (Chubb and Ewing, 1979) and testosterone biosynthesis requires the coordinated activity of several oxygen-dependent steroidogenic reactions (Ewing and Brown, 1977). Testosterone in the testicular venous effluent was determined with a specific antibody as previously described and validated for testes perfused in vitro (Chubb and Desjardins, 1983; Chubb and Draper, 1985).

Fluoride Ion Analysis

Fluoride ions in the media were quantified with a combination fluoride electrode (Catalog no. 960900; Orion Research Inc., Cambridge, MA) and a Microprocessor Ionalyzer (Model 901; Orion Research). The electrode is specific for ionic fluoride, which is the physiologically active form of fluoride (Cremer and Büttner, 1970).

Statistical Procedures

The significance of the difference between means was determined by Student's *t*-test. All results are expressed as mean ± SEM.

RESULTS

Testosterone Secretion

Testes perfused with medium incorporating untreated Oxypherol-E.T. secreted significantly less ($P<0.001$) testosterone than the contralateral testes perfused with medium incorporating treated Oxypherol-E.T. (Table 2). Perfusion flow rate and pressure were similar in all perfusions.

TABLE 1. Components of the perfusion media.

	Percentage (wt/vol)
Perfluorotributylamine (FC-43)	20
Pluronic F-68	2.6
NaCl	0.6
KCl	0.03
$MgSO_4 \cdot 7H_2O$	0.03
$CaCl_2$	0.02
KH_2PO_4	0.02
$NaHCO_3$	0.04
Penicillin	0.06
HEPES[a]	0.6
BSA[b]	3.0

[a] *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid.

[b] Bovine serum albumin, Fraction V (A-9647, Sigma Chemical, St. Louis, MO).

TABLE 2. Testosterone secretion by rat testes perfused in vitro.

Oxygen carrier	Testosterone secretion [ng/(g testis•h)]
Untreated Oxypherol-E.T.	390 ± 74 (n=4)
Treated Oxypherol-E.T.	1902 ± 104 (n=4)

Toxic Contaminants

The major difference between the two perfusion media was the concentration of fluoride ions. The untreated emulsion contained 3.7 ppm fluoride ions. In contrast, the resin dialysis-treated perfluorochemical emulsion had a minimal level (0.1 ppm) of fluoride ions. Fluoride ion concentration was effectively lowered by either resin treatment or dialysis. However, the combined treatment has been adopted as standard procedure for emulsion prepared in our laboratory.

Additional experiments were performed to determine if fluoride ions were the toxic contaminant in the Oxypherol-E.T. emulsion. In these experiments, LH-stimulated rat testes were perfused with a perfluorochemical medium containing different concentrations of fluoride ions. The results (Fig. 1) demonstrate that 3.3 ppm fluoride ions significantly depressed ($P<0.05$) testosterone secretion. Importantly, testes perfused with medium containing 3.3 ppm fluoride ions or untreated Oxypherol-E.T. secreted testosterone at similar rates.

DISCUSSION

The results demonstrate for the first time that the endocrine toxicity of Oxypherol-E.T. can be reversed by treatment with resin and dialysis. Testosterone secretion by rat testes is similar whether the perfusion medium incorporates treated Oxypherol-E.T. or erythrocytes as oxygen carriers (see Chubb and Draper, 1985). The experimental data support fluoride ions as

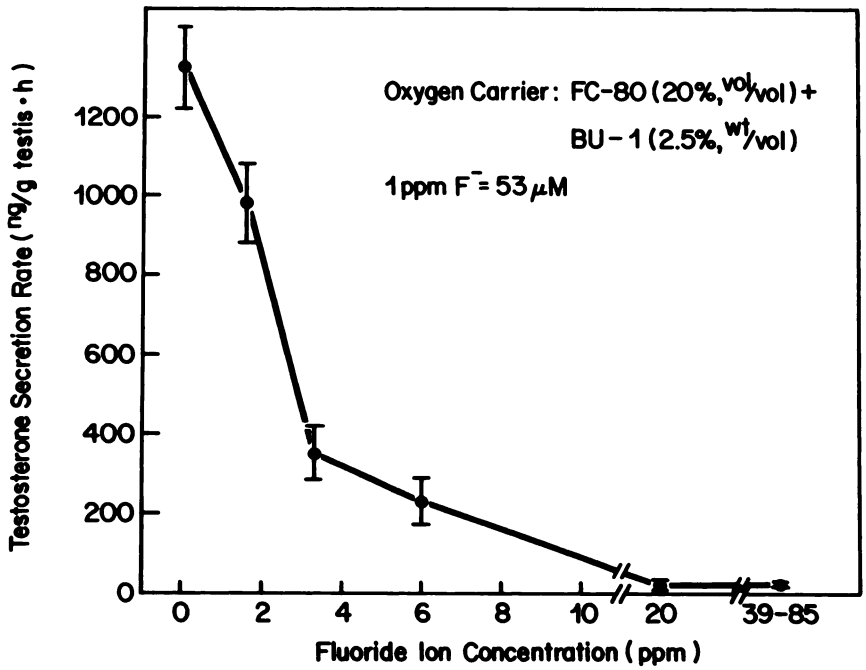

FIG. 1. Testosterone secretion by rat testes perfused in vitro for 3 h with perfluorochemical medium containing varying concentrations of fluoride ions. Venous effluent from LH-stimulated rat testes was analyzed radioimmunometrically for testosterone. The perfluorochemical medium was produced in our laboratory by a previously described procedure (Chubb and Draper, 1985). The medium composition was identical to that displayed in Table 1 except the medium contained 40% (wt/vol) of perfluoro-2-butyltetrahydrofuran (FC-80; PCR Research Chem, Gainesville, FL) and 2.5% (wt/vol) Butronic Polyol U-1 (a gift from Dr. I. R. Schmolka, BASF Wyandotte, Wyandotte, MI) as the perfluorochemical emulsion. Fluoride ions were added as sodium fluoride (Sigma). Each point represents the mean ± SEM of values for 3–5 animals.

the primary toxic contaminant of untreated Oxypherol-E.T.

Toxicity of Oxypherol-E.T. is not an isolated phenomenon. For example, six different lots of Oxypherol-E.T. purchased during our initial studies of perfluorochemicals inhibited testosterone secretion by rat testes perfused in vitro (Chubb and Draper, 1985). Similar negative results were obtained when Oxypherol-E.T. was mixed with the annex solutions provided by Green Cross; testosterone secretion was 481 ± 57 ng/(g testis·h) (n=3). Together, these data suggest that the endocrine toxicity of Oxypherol-E.T. is a continuing problem.

Although certain nonendocrinologic experiments involving Oxypherol-E.T. have been successful (Schneeberger and Neary, 1982; Tomera and Geyer, 1982), other experiments have failed (Bucala et al., 1984; Fournier et al., 1980). Geyer (1979) has reported that the toxicity of Oxypherol-E.T. varied with different batches when tested in rat exchange perfusions. Consequently, the toxicity of Oxypherol-E.T. appears to be variable and may be related to the time between manufacture and actual use.

Green Cross pamphlets describing Oxypherol-E.T. (Technical Information Ser. No. 3 dated 4 September 1976 and Ser. No. 5 dated 1 July 1981) state that Oxypherol-E.T. is a uniform emulsion of high purity perfluorochemicals that remains stable for 2 or 3 yr at 2–10°C. Stability is defined only on the basis of particle size. Toxicity associated with storage time is not discussed. In our previously reported studies (Chubb and Draper, 1985), Oxypherol-E.T. was used 9–13 mo after the date of manufacture. The date of manufacture had been replaced by an expiration date on the Oxypherol-E.T. purchased for this study. Preliminary experiments (C. Chubb, unpublished observations) have demonstrated that FC-43 emulsions prepared in our laboratory become toxic after 1 mo of storage at 4°C. Fluoride ion concentration increased from 0.2 to 0.8 ppm in these emulsions. The endocrine toxicity could be reversed by the clean-up procedure described in this report. The toxicity could stem from fluoride ions or unidentified breakdown products of the perfluorochemicals or emulsifiers.

Clark and coworkers (1972, 1975) identified fluoride ions as deleterious by-products of perfluorochemical emulsions prepared by sonication. However, Oxypherol-E.T. emulsion is prepared by a mechanical homogenization process that is supposed to eliminate the release of fluoride ions (Clark et al., 1974). The amount of fluoride ions liberated during Oxypherol-E.T. emulsification has not been reported. The origin of the fluoride ions in Oxypherol-E.T. is not understood since the F–C bond is highly stable with a bond energy of 486 kJ/mol (Flutec Handbook; I.S.C. Chemicals Limited, Bristol, England).

Fluoride ion has been reported to block cellular activation by LH (Ascoli and Puett, 1978; Kanwar et al., 1983). Kanwar and coinvestigators (1983) reported that 100 ppm fluoride ions significantly inhibited testosterone production by incubated rat testes. Our studies indicate that 3 ppm fluoride ions significantly inhibit testosterone secretion by rat testes perfused in vitro. Fluoride has also been reported to inhibit cellular activation by glucagon (Harwood and Rodbell, 1973) and thyroid-stimulating hormone (Bürgi et al., 1984). Investigators have theorized that the fluoride ion blunts the cAMP response to hormonal stimulation (Kanwar et al., 1983) or disrupts cofactor metabolism (Ascoli and Puett, 1978). At present, the mechanism by which fluoride ion blocks hormone action or testicular steroidogenesis remains unknown.

In conclusion, Oxypherol-E.T. contains contaminants that are toxic to endocrine organs. Fluoride ion may be the primary endocrine toxicant. The removal of the toxic contaminants by resin-dialysis treatment produced a uniform perfluorochemical emulsion that effectively transports oxygen to steroidogenic reactions.

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