33. IN VITRO DECONTAMINATION OF COBALT-60 EXPOSED PIGS EYES WITH DIPHOTERINE® VS. WATER

Patrick Gerasimo*, Pierre LaRoche**, Laurence Mathieu+, Joel Blomet+, Alan H. Hall#
*Laboratoire de Controle Radiotoxicologique, Service de Protection Radiologique de Armees, 1 bis, rue du Lt. Raoul-Batany, 92141 Clamart Cedex, France
** Arsenal de Brest, Brest, France
+Laboratoire PREVOR, Moulin de Verville, F95760, Valmondois, France
#Department of Emergency Medicine, Division of Toxicology, Texas Tech University Heath Sciences Center-El Paso, El Paso, TX, USA and TCMTS, Inc., 3456 Oxcart Run Street, El Paso, TX, 79936, USA

ABSTRACT

Introduction: Diphoterine® is widely used in Europe to decontaminate occupational eye/skin chemical splashes. Previous in vitro experiments demonstrated efficacy of Diphoterine® decontamination of human skin fragments exposed to C14-labeled Sulfur Mustard (Agent HD), Uranium-238, Cesium-137, and Strontium/Yttrium-90. In fact, Diphoterine® decontaminates over 600 chemical substances including sulfur mustard (Agent HD) in vitro and in human skin fragments in vitro. A list of the chemical substances against which Diphoterine® has been tested can be found at www.prevor.com (the manufacturer is currently conducting negotiations to have the product further tested against sulfur mustard and organophosphate nerve agents in the US.) It is essentially nontoxic (LD_{50}s > 2,000 mg/kg by oral or ingestion routes in rodents), nonirritating to the skin or eyes in experimental animals and humans, and its decontamination products are not toxic to the environment. Materials and Methods: These studies were conducted in the French Army Laboratory for Radiotoxicological Control. Pig eyes were obtained from an abattoir, exposed in vitro for 3 minutes to 20 microliters of a Cobalt-60 (120 Bq) source (LMRI, Orsay, France), and radioactivity was measured by gamma emissions (Germanium HP, Canberra). Radioactivity penetration was measured in control eyes. Following exposure, eyes were decontaminated with water or Diphoterine® (PREVOR, Valmondois, France): 1) 150 mL over 3 minutes begun at 3, 10, or 90 minutes; 2) 3 separate 150 mL over 3 minutes lavages; 3) eyewash lavage with 100 mL over 1 or 5 minutes. Lavage fluid residual radioactivity was measured a mean of 5 or 6 times (variation </= 1%). Results: After 3 minutes of in vitro exposure of pig eyes to Cobalt-60, in controls > 98% of the measurable radioactivity remained on the cornea, 10 minutes later > 10% penetrated into the aqueous humor, and at 90 minutes > 45% penetrated into the aqueous humor and about 5% penetrated into the vitreous humor. With all 3 lavage protocols, Diphoterine® was significantly more efficacious than water for eye decontamination in this model ($p < 0.05$). Summary: with chelating and hypertonic properties was superior to water for decontamination of Cobalt-60 eye exposure.

INTRODUCTION

Diphoterine®is an hypertonic, polyvalent, amphoteric compound for eye/skin chemical splash decontamination. *In vitro* and *in vivo*, it decontaminates approximately 600 chemicals, including acids, alkalis, oxidizing and reducing agents, irritants, lacrimators, solvents, alkylating agents such as sulfur mustard, and radionuclides (U-238, Cs-137, Sr/Y-90). Its chemical bond energy for such agents is greater than that of tissue receptors. Its hypertonicity impedes chemical tissue penetration and may remove some amount of

skin/cornea-absorbed toxicants not bound to tissue receptors. Diphoterine chemical reactions are not exothermic. In experimental animals, Diphoterine® and its acid/alkali decontamination residues were not irritating to the eyes or skin. It is essentially nontoxic (LD_{50}'s > 2000 mg/kg by oral and dermal exposure routes in rodents).

In human volunteers, Diphoterine® did not irritate the eyes. Diphoterine® has prevented or decreased the severity of chemical eye/skin burns with 96% sulfuric acid, 100% acrylic acid, 50% acrylamide, solid sodium hydroxide flakes, and dimethylethylamine. In more than 600 workers in 4 European workplaces, Diphoterine® decontamination of acid and base chemical splashes was associated with significant decreases in lost work time and the need for additional burn treatment as compared with water lavage. Diphoterine® is an efficacious decontamination product for eye/skin chemical splashes. It washes harmful chemicals off exposed tissues as well as actively neutralizing them.

The purpose of the experiments reported here was to compare the effects of Diphoterine® versus water for decontamination of cobalt-60 in pigs eyes in vitro, thus comparing an active hypertonic and chelating decontamination fluid with the simple mechanical rinsing activity of water.

MATERIALS AND METHODS

These studies were conducted in the French Army Laboratory for Radiotoxicological Control, Clamart, France. Pig eyes were obtained from an abattoir just after slaughter, exposed in vitro for 3 minutes to 20 microliters of a standard Cobalt-60 (120 Bq) source (LMRI, Orsay, France), and radioactivity was measured by gamma emissions (Germanium HP, Canberra). Radioactivity penetration was measured in the cornea, aqueous humor, and vitreous humor of control eyes.

Following Cobalt-60 exposure for 3 minutes, test eyes were decontaminated with water or Diphoterine® (PREVOR, Valmondois, France) using 3 protocols: 1) 150 mL lavage over 3 minutes begun at 3, 10, or 90 minutes after exposure; 2) 3 separate successive 150 mL lavages over 3 minutes each as above; or, 3) bathing in an eyewash device with 100 mL over 1 or 5 minutes. Lavage fluid residual radioactivity was measured a mean of 5 or 6 times (variation </= 1%) and the Mean and Standard Error of the Mean were calculated for both the amount or radioactivity in the residual lavage fluid (in Bq) and the percentage of radioactivity removed.

RESULTS

A single lavage after a delay of 90 minutes following contamination: 150 mL of lavage fluid utilized (water or Diphoterine®) See Table 1. Distribution of radioactivity in control eyes at 90 minutes after contamination. There was little effect of lavage as the delay was too long. The radio-element had time to diffuse into the interior of the eye and become bound to tissues in an irreversible fashion.

A single lavage following a delay of 10 minutes following contamination: 150 mL of lavage fluid utilized (water or Diphoterine®). See Table 2. Distribution of radioactivity in control eyes at 10 minutes after contamination

Efficacy limitation of lavage: a part of the radioactivity is accessible to lavage. There was a tendency for Diphoterine® to be shown superior to water.

A single lavage following a delay of 3 minutes after contamination (minimal time to effect decontamination if the decontamination product is pre-placed at the accident site): 150

mL of lavage fluid utilized (water or Diphoterine®). See Table 3. Distribution of radioactivity following a delay of 3 minutes after contamination.

The lavages were efficacious. The performance of three successive lavages with water gave results on the same order as those with a single water lavage. The performance of three successive lavages with Diphoterine® gave clearly better results than with a single lavage as this molecule binds the Cobalt-60 due to its hypertonic and chelating properties.

Bathing the cornea with the aid of an eyewash device (containing 100 mL) following a delay of 3 minutes between contamination and treatment. The duration of bathing was 1 minute.

Number of trials to calculate the Mean and the SEM: n = 6.

Radioactivity of lavage with water = 41.13 +/- 2.09; % of radioactivity removed = 34.28+/- 1.74.

Radioactivity of lavage with Diphoterine® = 81.30 +/- 7.10; % of radioactivity removed= 51.08 +/- 6.92 ($p<0.05$).

Bathing with Diphoterine® is more efficacious than bathing with water, but the results are slightly worse than those obtained with three successive lavages because there is not the effect of mechanical rinsing.

Bathing the cornea with the aid of an eyewash device (containing 100 mL) following a delay of 3 minutes between contamination and treatment. The duration of bathing was 5 minutes.

Number of trials to calculate the Mean and the SEM: n = 6.

Radioactivity of lavage with water = 59.48 +/- 2.43; % of radioactivity removed = 49.67+/- 2.03.

Radioactivity of lavage with Diphoterine® = 90.92 +/- 3.29; % of radioactivity removed= 76.77 +/- 2.74 ($p<0.05$). Bathing with Diphoterine® was more efficacious than bathing with water.

CONCLUSIONS

It is necessary to intervene rapidly because Cobalt-60 rapidly diffuses into the interior of the eye and this a part of the total amount of the radio-element is nor accessible to surface decontamination fluids. For this reason, the treatment device should be pre-placed at locations with contamination risk, close to hand.

Diphoterine® used in the form of an eyewash was shown to be much more efficacious that water. In our experimental conditions, lavage with water can only remove about 40% of the radioactivity, while about 60% can be removed with Diphoterine®. When Diphoterine® is used in an eyewash device, it is possible to remove about 75% of the radioactivity. A specific product such as Diphoterine®, which has both hypertonic and chelating properties is this superior to simple lavage with water.

REFERENCES

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KEY WORDS
Diphoterine®, eye decontamination, Cobalt-60, radionuclide, decontamination solutions

FIGURES AND TABLES

Table 1. Distribution of radioactivity in control eyes at 90 minutes after contamination

Part of the Eye	Radioactivity in Bq/Sample	Distribution in %
Cornea	39.84 +/- 3.41	48.1 +/- 4.13
Aqueous Humor	37.87 +/- 3.29	46.0 +/- 3.77
Vitreous Humor	4.91 +/- 0.65	5.0 +/- 0.79

Half of the radioactivity diffused into the interior of the eye.
Number of trials to calculate the Mean and the Standard Error of the Mean (SEM): n = 6.
Radioactivity of lavage with water = 9.44 +/- 2.36; % of radioactivity removed = 7.87 +/- 1.98
Radioactivity of lavage with Diphoterine® = 11.20 +/- 1.95; % of radioactivity removed = 8.33 +/- 1.63.

Table 2. Distribution of radioactivity in control eyes at 10 minutes after contamination

Part of the Eye	Radioactivity in Bq/Sample	Distribution in %
Cornea	78.41 +/- 8.54	86.24 +/- 9.39
Aqueous Humor	8.55 +/- 1.78	10.40 +/- 1.94
Vitreous Humor	3.12 +/- 1.75	3.42 +/- 1.28

The radioactivity was mainly localized on the cornea, but a small part had begun to diffuse into the interior of the eye.
Radioactivity of lavage with water = 19.85 +/- 2.58; % of radioactivity removed = 18.64 +/- 2.13.
Radioactivity of lavage with Diphoterine® = 28.80 +/- 5.53; % of radioactivity removed = 22.33 +/- 4.61.

Table 3. Distribution of radioactivity following a delay of 3 minutes after contamination.

Part of the Eye	Radioactivity in Bq/Sample	Distribution in %
Cornea	89.17 +/- 7.12	88.57 +/- 7.87
Aqueous Humor	0.83 +/- 0.48	0.92 +/- 0.53
Vitreous Humor	0.48 +/- 0.43	0.51 +/- 0.48

Number of trials to calculate the Mean and the SEM: n = 5.
Radioactivity of lavage with water = 53.15 +/- 7.31; % of radioactivity removed = 44.29 +/- 6.09.
Radioactivity of lavage with Diphoterine® = 58.45 +/- 1.04; % of radioactivity removed = 48.72 +/- 0.87.
Lavage was efficacious. There was little difference between water and Diphoterine®.

Three separate successive lavages following a delay of 3 minutes after contamination: 150 mL of lavage fluid utilized (water or Diphoterine®). This protocol corresponds to that applied in cases of external radiation contamination.
Number of trials to calculate the Mean and the SEM: n = 5.
Radioactivity of lavage with water = 52.08 +/- 3.78; % of radioactivity removed = 43.40 +/- 3.15.
Radioactivity of lavage with Diphoterine® = 71.44 +/- 8.49; % of radioactivity Removed = 59.63 +/- 5.41 ($p<0.05$).