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Effects of a desealant formulation, SR-51® and its individual components on the oxidative functions of mitochondria

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Abstract

The Royal Australian Air Force has reported that personnel involved in F-111 fuel tank maintenance were concerned that exposure to a range of chemicals during the period 1977–mid-1990s was the cause of health problems. Particular concern was directed at a desealant chemical mixture known as SR-51®. The current study, using in vitro submitochondrial assays, was designed to investigate the relative toxicities of the four components of SR-51® (Aromatic 150 solvent (Aro150), dimethylacetamide (DMA), thiophenol (TP) and triethylphosphate (TEP)). Based on the EC_{50} values, TP and Aro150 were the most toxic components and were markedly more toxic than TEP and DMA.

Keywords: Submitochondrial particles; Solvents; In vitro toxicity

1. Introduction

Toxicological investigations usually examine the effects that are caused by single chemicals. However, most chemical products, such as industrial desealants, are formulations consisting of several chemicals. A recent investigation undertaken by the Royal Australian Air Force (RAAF) reported that a number of their personnel involved in F-111 fuel tank maintenance were concerned that occupational exposure to a range of chemicals during the period 1977–mid-1990s was the cause of past and current health problems (RAAF, 2001).

The maintenance workers complained of headaches, skin rashes, memory loss and various neurological symptoms (RAAF, 2001). These complaints were almost entirely ignored by the commanding officers and medical staff. It was not until September 1999, more than 22 years later – when a new sergeant took charge of the fuel tank maintenance section – that any action was taken. The new officer encouraged all affected personnel to see a doctor at the medical centre (RAAF, 2001). At the same time medical staff noticed that protective gear used in the program was inadequate. By the end of January 2000 the commanding officer of the Aircraft Maintenance Squadron was contacted about the problems and the spray seal program was suspended (RAAF, 2001). A major health study of personnel involved in the fuel maintenance program is currently underway in an attempt to characterise whether an association exists between the past and current health status of the personnel and their occupational chemical exposure during their involvement in the fuel maintenance program. One of the chemical formulations of concern was a desealant formulation, SR-51®. SR-51® consists of four components: 75% Aromatic 150 solvent (Solvesso 150), 10% dimethylacetamide (DMA), 10% thiophenol (TP) and 5% triethylphosphate (TEP). This study examined the toxicity of SR-51® using an in vitro screening assay, the submitochondrial particle (SMP) assay (Blondin et al., 1989; Knobeloch et al., 1990). The complete formulation as well as its individual components were tested. Submitochondrial particles (SMPs) consist of inverted, vesicular portions of the inner mitochondrial membrane, which retain the capacity

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to perform the integrated oxidative functions of intact mitochondria. The assay consists of two tests, the electron transfer reaction (ETR) and the reverse electron transfer (RET) reaction that both measure the concentration of the test chemical that causes a 50% inhibition in the activity of the SMPs (i.e. an EC_{50}).

2. Materials and methods

2.1. The submitochondrial particle (SMP) assays

SMPs (40 mg/ml) were purchased from MitoScan Corp., Madison, USA. A modified spectrophotometric method of Blondin et al. (1989) measured the rate of NADH oxidation in the ETR assay and NAD reduction in the RET assay at 340 nm. Details of the modified assays are described by Oakes and Pollak (1999). A control rate of NADH oxidation and NAD reduction of 0.02 absorbance units/min (i.e. 3.22 nmol NADH/min) was used in both in the ETR and RET assays. Under the assay conditions this corresponded to a basal rate of NADH oxidation for the ETR assay of 0.08 μmol/mg SMP/min and a basal rate NAD reduction for the RET assay of 0.04 μmol/mg SMP/min.

2.2. SR-51® and its components

SR-51® was prepared according to archival specifications provided by of the manufacturer, the Eldorado Chemical Company, Texas, USA (the company ceased production of SR-51® in the mid-1980s). SR-51® consisted of four components: an aromatic solvent, Solveso 150 (Aro150), dimethyl acetamide (DMA), thiophenol (TP), triethyl phosphate (TEP). Solvesso 150 was kindly donated by Exxon Chemicals, Australia. DMA, TP and TEP were all purchased from Sigma Aldrich, Australia. To prepare SR-51®, the four components were mixed in the following ratio, Aro150:DMA:TP:TEP (15:2:2:1).

The experimental design was that the chemical mixture SR-51® would be tested in the assay and the concentration that caused a 50% reduction in ETR and RET activity (EC_{50}) would be determined. It was then proposed to test each component chemical of the mixture and determine their EC_{50} to establish their individual toxicity; SR-51®. The components were diluted with dimethyl sulfoxide (DMSO) for the use in the SMP assay. DMSO was found not to inhibit SMP activity at the maximal concentration (0.005%) used in the assay.

A preliminary study had to be performed to determine the concentration range of SR-51® and its components for the final assay. This was done by performing 1 in 10 serial dilutions of SR-51® in DMSO and using these concentrations for preliminary testing. The concentration range chosen for the final assay were concentrations of SR-51® that inhibited SMP activity within the range 15–85%. Preliminary testing to determine the assay concentration range was performed for each of the components of SR-51®. All assays were repeated four times.

Table 1
EC_{50} (the effective concentration that causes 50% inhibition of submitochondrial particle (SMP) activity) of SR-51 and its components using the ETR and RET assays

	EC_{50} (μg/ml)	
	ETR assay	RET assay
SR-51	16.4 ± 1.5	15.3 ± 1.9
Thiophenol	3.3 ± 0.2	7.32 ±
Aromatic solvent	20.0 ± 0.7	11.1 ± 1.1
Triethyl phosphate	7700 ± 558	1800 ± 223
Dimethyl acetamide	59670 ± 2270	18400 ± 1151

2.3. Partial formulations of SR-51®

Partial formulations of SR-51® were also tested in the RET and ETR assays to determine the contribution to the toxicity of SR-51® by its individual components. Partial formulations consisted of all of the ingredients of the original formulations, except for one, which was replaced with DMSO. Four partial formulations were tested: SR-51® minus Aro150, SR-51® minus DMA, SR-51® minus TP and SR-51® minus TEP. A concentration of SR-51® (25 μg/ml) was chosen for testing all partial formulations; the concentration of SR-51® that inhibited SMP activity by 60–65%. All partial formulations were assayed simultaneously, together with the complete formulation and a negative control (0.005% DMSO).

2.4. Statistics

Data was collected from four independent experiments. For each experiment an EC_{50} value was calculated using line regression analyses. The mean and standard deviation of the EC_{50} values (n = 4) were then determined. For the experiments using partial formulations, the rates of inhibition of SMP activity were compared to the complete formulation of SR-51® for statistical significance using the Student's t-test. $P < 0.05$ was considered significant.

3. Results

3.1. ETR and RET assays

3.1.1. SR-51®

In both the ETR and RET assays, SR-51® caused a concentration-dependent inhibition of the rate of NADH oxidation. The mean EC_{50}s (n = 4) for SR-51® were 16.4 ± 1.5 and 15.3 ± 1.9 μg/ml, respectively (Table 1).

3.1.2. Individual components of SR-51®

The four component chemicals of SR-51® were tested individually and the calculated EC_{50}s are shown in Table 1. The EC_{50}s of TP and Aro150 in the both the ETR and RET

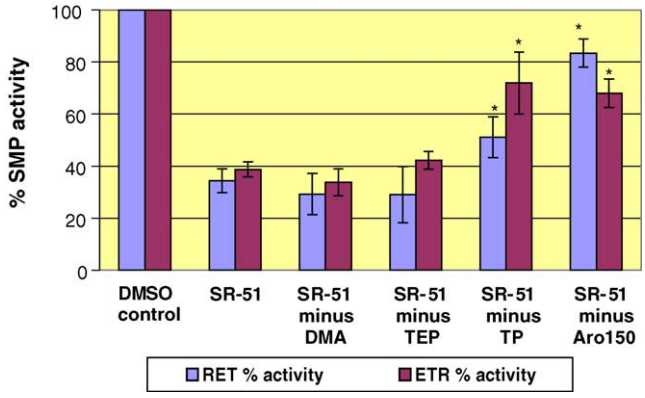

Fig. 1. Comparison of the toxicity of SR-51 and its components using the submitochondrial particle (SMP) assays, the RET and the ETR test. The toxicity of complete and partial formulations of SR-51 was compared using both the RET and ETR assays. A single concentration of 25 μg/ml (causes 60–65% inhibition of SMP activity) was chosen to compare the toxicity of the formulations. Dimethyl sulphoxide (DMSO) was used as the vehicle control. The standard deviation is shown by the bars. Results were analysed using a paired *t*-test at $P < 0.05$. A statistical difference ($P < 0.05$) between the partial formulations compared to the complete SR-51 formulation is indicated by an asterisk (*).

assays were markedly lower than those obtained using either TEP or DMA.

3.2. *Partial formulations of SR-51®*

In order to ascertain the contribution of the individual components to the toxicity of the complete formulation of SR-51®, partial formulations were also tested in both the ETR and RET assay. In the ETR assay, the results showed that the SMP toxicity of 25 μg/ml SR-51® was significantly reduced ($P < 0.05$) when tested as a partial formulation without Aro150 or a partial formulation without TP (Fig. 1). In the absence of DMA or TEP, however, the toxicity of 25 μg/ml SR-51 remained unchanged.

Similar results were obtained using the RET assay, partial formulations of SR-51® demonstrated that in the absence of the Aro150 or TP component, SR-51® was significantly less toxic to SMPs. In the absence of DMA or TEP, however, the toxicity of 25 μg/ml SR-51 remained unchanged (Fig. 1).

4. Discussion

The present study demonstrated that SR-51® formulation inhibited oxidative functions of SMPs in vitro. When components of SR-51® were tested separately, it was found that TP was the most toxic and Aro-150 was the second most toxic component (Table 1). The other two components, TEP and DMA did not appear to significantly contribute to the toxicity of the formulation. Results obtained using partial formulations of SR-51® confirmed that its toxic effect was mostly due to TP and Aro150 (Fig. 1).

The EC_{50} value for TP obtained in the SMP assays is markedly lower than the EC_{50} determined for either DMA or TEP. Published in vivo studies confirm this toxicity rating. The reported oral LD_{50} value in rats for thiophenol (42.6 mg/kg) indicated it is at least 28-fold more toxic than TEP (1.1–1.6 g/kg) and over 100 times more toxic than DMA (4.9 g/kg) or aromatic petroleum solvents similar to Aro150 (4.7 g/kg) (Fairchild and Stockinger, 1958; Gosselin et al., 1984; Bayer Miles and Inc., 1995; IARC, 1989). There is some evidence that in bacterial assays toxicity of TP is reduced when it is tested with mammalian liver enzyme preparations (Lavoie et al., 1979). This suggests that in an in vivo situation TP may undergo metabolic inactivation in the liver. Studies in rats orally dosed with TP (6 mg/kg) show that almost half of the TP (45%) is converted to less toxic metabolites and excreted in urine by 60 h post-administration (McBain and Menn, 1969). TP would therefore be expected to appear more toxic in an in vitro system such as SMP, where mammalian metabolic enzymes are not used.

The relatively high toxicity of the Aro150 in the SMP test compared to TEP and DMA, however, is not reflected in published in vivo studies. Based on oral LD_{50} obtained in rats, aromatic petroleum solvents such as Aro150 (4700 mg/kg) have a similar LD_{50} to DMA (Fairchild and Stockinger, 1958; Gosselin et al., 1984; Bayer Miles and Inc., 1995; IARC, 1989).

Chemicals that inhibit functions of SMPs in both ETR and RET assays may belong to the class of Complex I inhibitors (Oakes and Pollak, 1999). Electrons have to flow through Complex I in both ETR and RET, therefore an inhibitor of this complex would reduce the observed activity of SMPs in both assays. It is also possible that SR-51® is not a specific inhibitor of any particular complex in the electron transport chain, but acts by disrupting the inner membrane of SMPs. The major component of SR-51® is an aromatic solvent, Aro150 (75% v/v). It is known that aromatic solvents produce disruptive effects on biological membranes (Sikkema et al., 1994, 1995; Bondy and McKee, 1991), hence a likely mechanism of SMP toxicity is disruption of the inner mitochondrial membrane of the SMPs.

The SMP test is an in vitro system that uses only a part of an organelle found in mammalian cells. Hence, it is unable to account for the metabolic activation or inactivation of the test chemical that may occur in vivo. It also does not account for the pharmacokinetic behaviour of the tested chemical. Although the SMP assay is useful for the preliminary evaluation of toxicity of chemical agents, complementary studies of the pharmacokinetics of the tested chemicals have to be conducted to further evaluate toxicity.

It is unknown if the intracellular concentration of these chemicals or the formulation in a dosed animal could reach concentrations similar to the EC_{50}s determined in this assay. Further in vivo studies of SR-51® are required to evaluate toxicity of the formulation. These studies should include the determination of blood levels of the SR-51® components in the dosed animal, which would help determine if the blood

levels of the chemical mixture are likely to reach the EC_{50} concentration found in this assay, and therefore allow an assessment of whether SR-51® is likely to produce toxic effects on mitochondria in vivo.

In conclusion, the reported results have established that SR-51® and its component chemicals are all capable of interfering with in vitro mitochondrial function. Aro150 and TP are the most toxic components of the mixture based on their relatively low EC_{50}s compared to those obtained for DMA and TEP.

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