The percutaneous absorption of soman in a damaged skin porcine model and the evaluation of WoundStat™ as a topical decontaminant

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Purpose: The aim of this study was to evaluate a candidate haemostat (WoundStat™), down-selected from previous *in vitro* studies, for efficacy as a potential skin decontaminant against the chemical warfare agent pinacoyl methylfluorophosphonate (Soman, GD) using an *in vivo* pig model.

Materials and methods: An area of approximately 3 cm^2 was dermatomed from the dorsal ear skin to a nominal depth of 100 μm. A discrete droplet of ^{14}C-GD (300 μg kg^{-1}) was applied directly onto the surface of the damaged skin at the centre of the dosing site. Animals assigned to the treatment group were given a 2 g application of WoundStat™ 30 s after GD challenge. The decontamination efficacy of WoundStat™ against GD was measured by the direct quantification of the distribution of ^{14}C-GD, as well as routine determination of whole blood cholinesterase and physiological measurements.

Results: WoundStat™ sequestered approximately 70% of the applied ^{14}C-GD. Internal radiolabel recovery from treated animals was approximately 1% of the initially applied dose. Whole blood cholinesterase levels decreased to less than 10% of the original value by 15 minutes post WoundStat™ treatment and gradually decreased until the onset of apnoea or until euthanasia. All treated animals showed signs of GD intoxication that could be grouped into early (mastication, fasciculations and tremor), intermediate (miosis, salivation and nasal secretions) and late onset (lacrimation, body spasm and apnoea) effects. Two of the six WoundStat™ treated animals survived the study duration.

Conclusions: The current study has shown that the use of WoundStat™ as a decontaminant on damaged pig ear skin was unable to fully protect against GD toxicity. Importantly, the findings indicate that the use of WoundStat™ in GD contaminated wounds would not exacerbate GD toxicity. These data suggest that absorbent haemostatic products may offer some limited functionality as wound decontaminants.

Keywords: soman, chemical warfare agent, decontamination, contaminated wound, WoundStat™.

Introduction

Military personnel are constantly exposed to the risk of traumatic, penetrating injury on the battlefield (1-4) and various haemostatic treatments have been evaluated in combat settings (5-8). Should wounding occur in a chemically contaminated environment, such as after hostile deployment of a chemical warfare agent (CWA), the use of haemostatic treatments would be complicated by the need to decontaminate the wound site. Of major concern is whether the use of a haemostat on a chemically contaminated wound would increase systemic absorption. Rather than using separate strategies to arrest haemorrhage and decontaminate a wound site, the development of a product that simultaneously arrests haemorrhage and decontaminates wounds would have clear advantages. Studies involving the use of CWAs to evaluate the efficacy of potential medical countermeasures, by necessity, use animal models to indicate what would happen in humans. For studies involving the dermal exposure route, the pig is generally accepted as being the most representative model for man (9-10).

Previous *in vitro* studies have demonstrated that haemostatic products may retain the ability to clot blood in the presence of CWAs and that certain products (based on absorptive powders) are able to effectively decontaminate CWAs from normal and superficially-damaged skin (11-13). One product, WoundStat™, was identified as being particularly effective in terms of its ability to decontaminate CWAs from undamaged or superficially-damaged skin. The purpose of the current study was to extend the evaluation of WoundStat™ to an *in vivo*, non-haemorrhaging wound model using the CWA soman (pinacoyl methylfluorophosphonate).

Methods

Chemicals

The synthesis, use and destruction of soman (GD; pinacoyl methylfluorophosphonate) was conducted in accordance with the Chemical Weapons Convention (1996). Radiolabelled GD was synthesised by TNO (Rijswijk, Netherlands) and had a radiochemical purity >97% (as determined by radiometric HPLC analysis). The chemical purity of unlabelled GD (supplied by Dstl Detection Department, Salisbury, Wiltshire) was reported to be >97% (as measured by nuclear magnetic resonance spectroscopy). Radiolabelled and unlabelled GD were mixed in appropriate proportions to give a nominal activity of approximately 5 µCi µL^{-1}. Isofluorane-VET® (isofluorane; Merial Animal Health Ltd, Essex, UK), Hypnovel® (midazolam hydrochloride (5 mg mL^{-1}); Roche Products Ltd., Hertfordshire, UK), Dolethal® (sodium pentobarbitone (200 mg mL^{-1}); Schering-Plough, Hertfordshire, UK) were purchased from a registered UK supplier. Medical grade oxygen and nitrous oxide were obtained from BOC Ltd. (Surrey, UK). Liquid scintillation counting (LSC) materials (Soluene-350, Ultima Gold and opaque plastic vials) were purchased from Perkin-Elmer (Chandler's Ford, Hampshire). All other chemicals were analytical grade and were purchased from the Sigma Chemical Company (Poole, Dorset).

Treatment

The haemostat WoundStat™ was purchased from TraumaCure, Inc. (Bethesda, MD).

Animal model

The use of animals was in accordance with the Animals (Scientific Procedures) Act 1986. A total of 18 female weanling pigs (large white strain, weight range 15-25 kg) were purchased from a reputable supplier. Animals were given 24-h access to food and water. After a

minimum of one week's acclimatisation, an individual animal was prepared for experiment according to the following protocol.

Each animal was fasted overnight and then sedated with Hypnovel® (Midazolam, 6mL i.m., 5 mg mL^{-1}) prior to induction of gaseous anaesthesia (1.5-3% isofluorane delivered in 4-6 L min^{-1} O$_2$ and 0.4-0.6 L min^{-1} N$_2$O) via a nose cone. After the animal was fully anaesthetised, an endotracheal tube was inserted. Respiratory anaesthesia (as described above) was maintained via the endotracheal tube until completion of surgical preparation. To enable arterial and venous access, the internal carotid artery and jugular vein were cannulated. Upon completion of surgery, intravenous administration of Alfaxan (Alphaxalone, Astra Zeneca) commenced and 5 minutes later respiratory anaesthesia was discontinued. Alfaxan was delivered at a rate of 12 to 26 mL h^{-1} for the rest of the study. The general wellbeing of each animal was monitored continuously under anaesthesia using a Propaq vital signs monitor (Welch Allyn, Oregon USA). Parameters measured were arterial blood pressure, breathing rate, core temperature, CO$_2$, ECG, pulse rate and SpO$_2$. After a 30-minute stabilisation period, the animal was placed into a sling. The animal and sling were moved carefully into a fume cupboard. The left ear of the animal was secured in a horizontal position, with the outer ear being made available for commencement of study procedures.

Experimental procedures

A total of 18 animals were used, with animals being assigned to either the treated (GD challenge, WoundStat™ treatment), control (no GD challenge, no WoundStat™ treatment) or untreated (GD challenge, no WoundStat™ treatment) group. An area of approximately 3 cm^2 was dermatomed (Humeca Model D42, Eurosurgical Ltd., Guildford, UK) from the dorsal ear skin to a nominal depth of 100 μm. Any transient bleeding that resulted from the procedure was removed with a saline-soaked cotton swab. A plastic dosing template was secured around

the edges of the dermatomed area using surgical glue (Vetbond™). Following a 30-minute baseline measurement period, a discrete droplet of ^{14}C-GD (300 µg kg^{-1}) was applied directly onto the surface of the damaged skin at the centre of the dosing template. Animals assigned to the treatment group were given a 2 g topical application of WoundStat™ directly to the exposure site 30 s after contamination with GD. Once placed, the WoundStat™ was left undisturbed for the duration of the study. Animals in the control (untreated) group were exposed to GD but not subject to the application of WoundStat™. Arterial blood samples were taken into both sodium EDTA tubes and sodium citrated tubes at regular intervals both pre and post GD challenge. Blood samples taken into sodium EDTA tubes were used for haematocrit quantification and analysis of whole blood cholinesterase activity. Blood samples taken into sodium citrate tubes were used for radiometric quantification.

Terminal procedures

Euthanasia was achieved by i.v. bolus of Dolethal® (sodium pentobarbitone; 6 mL, 200 mg mL^{-1}) at 6 h (surviving animals) or 15 minutes after the onset of apnoea. The dosed ear was then carefully removed and the decontaminant (where appropriate) removed from the skin surface and placed in 16 mL of scintillation counting fluid (Ultima Gold, PerkinElmer LAS (UK) Ltd., Buckinghamshire, UK). The dosing template was removed and placed in 20 mL isopropanol. The skin surface was swabbed with cotton wool (to remove any residual GD) and the swab was placed in 16 mL of isopropanol. The skin exposure site was excised, and the periphery and the central area of the dosing site were separated to enable determination of skin surface spread from the central dosing area. These skin samples were placed in 16 mL Soluene-350. Each animal was then exsanguinated prior to post-mortem examination. Major organs (brain, heart, kidney, liver, lung, pancreas and spleen) were removed, weighed and frozen for subsequent radiometric analysis.

Whole blood cholinesterase measurement

A modified Ellman assay (14) was used to quantify whole blood cholinesterase. Briefly, enzyme activity was measured via reaction of thiocholine with 5,5-dithiobis-(2 nitrobenzoic) acid (DTNB). Prior to analysis, samples of arterial whole blood had been stored at -20°C for at least a week in sodium EDTA tubes. A 25 µL sample of whole blood was made up to 5 mL in a pH 8.0 phosphate buffer. A 1 mL sample of the resulting blood solution was incubated at 30°C in a cuvette containing both acetylthiocholine iodide (1 mM) and DTNB (0.25 mM). The reaction, monitored at 412 nm, was measured over a 10-minute period using a Biochrom Ultrospec 6300 spectrophotometer. Appropriate blanks (substrate and tissue) were run simultaneously with the test samples and were subtracted from the test reaction.

Radiometric analysis

Vials containing the dosing chamber, skin surface swab, dissolved skin were stored at room temperature (with occasional shaking) for up to two weeks, after which aliquots (250 µL) were removed into 5 mL of scintillation fluid. Organ samples (approximately 0.1 g) were dissected in triplicate, placed into 2 mL Soluene within a glass scintillation vial and heated at 60ºC for a period of 4 hours. After cooling to room temperature, 20 mL of Ultima gold liquid scintillation fluid was added to each vial. The method described by Moore (15) was used for blood sample preparation, as follows: a blood sample (0.4 mL) was placed into a glass liquid scintillation vial. To this 1 mL of a Soluene and isopropanol (1:2 ratio) mixture was added whilst the vial was swirled gently. Each vial was then heated at 60ºC for 2 hours. After cooling, 0.5 mL of 30% hydrogen peroxide was added with gentle agitation until foaming had subsided. Vials were allowed to stand for a period of 30 minutes prior to being heated to 60ºC for a period of 30 minutes. After cooling for 15 minutes, 15 mL of Ultima gold liquid scintillation fluid was added to each vial. The amount of radioactivity in each sample was

measured using a PerkinElmer Tri-Carb liquid scintillation counter (Model 2810 TR), with the manufacturer's ^{14}C-quench curve library set to exclude single-photon (non-radioactive) events. The amount of radioactivity in each sample was converted to an amount of GD by comparison to standards (containing known quantities of ^{14}C-GD) prepared and measured simultaneously. The total amount of GD contained within each organ was calculated by reference to the total weight of the organ weighed at post mortem. The total amount of GD in the circulating blood volume was derived from the analysis of Bush (16), where the Total Blood Volume = $(161.48 \times \text{total animal weight}^{-0.297}) \times \text{total animal weight} / 1000$. Absorption of ^{14}C-GD was measured according to the appearance of radiolabel within the circulating blood volume at defined time points after ^{14}C-GD challenge to the dosing site.

Radiometric analyses were grouped as external quantification, local quantification and internal quantification. External quantification grouped the unabsorbed fraction of GD and included the portions of radioactivity remaining on the skin surface or sequestered into the WoundStat™ treatment. Local quantification grouped radioactivity recovery within the skin at the dosing site and at the periphery of the dosing site. Internal quantification grouped the portions of radioactivity located in the blood, liver, kidney, pancreas, spleen, heart, lung and brain. Any radioactivity unaccounted for was assumed to have either volatilised from the skin surface or to have been present in unsampled organs.

Statistical analysis

GraphPad Prism version 6.01 for Windows (GraphPad Software) was used for normality testing, statistical analysis and graphical presentation of the data. Data were assessed for normal distribution using a D'Agostino and Pearson omnibus normality test and a Gaussian non-linear regression curve fit. Significance was predefined at an alpha-level of 0.05. Survival fractions and survival curve were analysed using the Kaplan-Meier (log-rank)

method, and Gehan–Brislow–Wilcoxon tests. Comparisons across multiple experimental groups were performed using a non-parametric ANOVA (Kruskal–Wallis test) with Dunn's multiple comparisons post-test or a Mann–Whitney test (comparison of pre-exposure and final measurements within the same experimental group).

Results

Survival and gross clinical observations

Application of GD to damaged skin *in vivo* resulted in the rapid onset of multiple observable (Table 1) or physiological (Table 2) signs of nerve agent poisoning. In addition, only one of the animals in the GD-exposed untreated group survived to six hours post-exposure (Figure 1) and median survival time differed significantly between the control and GD-exposed untreated groups (Figure 2). Two animals in the GD-exposed WoundStat™-treated group survived until the end of the study period (Figure 1). Signs of nerve agent poisoning were also observed in these animals, although there tended to be a longer latency in the onset of signs when compared to the untreated group (Table 1). In addition, there was no significant difference in median survival time between the GD-exposed WoundStat™-treated group and the unexposed control group (Figure 2). All six animals in the control group survived the six-hour study duration and showed no sign of GD intoxication.

Toxicodynamics

The clinical signs observed in the GD-exposed untreated group were accompanied by a rapid depression of whole blood cholinesterase to less than 5% of baseline values by 15 minutes post exposure (Figure 3) and an increase in whole blood haematocrit (Figure 4). Similarly, the GD-exposed WoundStat™-treated group exhibited rapidly decreasing whole blood cholinesterase (Figure 3) and increased haematocrit (Figure 4) during the study.

Toxicokinetics

Blood levels of ^{14}C-GD reached a maximum within 10 minutes of GD challenge for both untreated and WoundStat™ treated groups (Figure 5). In the WoundStat™ treatment group, the amount of ^{14}C-GD in the blood had halved by 20 minutes post-exposure and remained constant at this level for the study duration. In contrast, in the untreated group, the amount of ^{14}C-GD in the blood steadily increased as the study progressed. Comparison of whole blood cholinesterase and the amount of ^{14}C-GD in the blood indicated that a blood recovery of >15 µg ^{14}C-GD resulted in maximum cholinesterase inhibition (Figure 6).

Distribution

Upon completion of the untreated studies, the majority of the applied ^{14}C-GD was located either on the skin surface or within the skin at the dosing site, while a smaller portion of radioactivity was recovered from the periphery of the dosing site (Figure 7a-d). In comparison, for the treated animals the majority (approximately 70%) of the applied ^{14}C-GD was sequestered by Woundstat™ (Figure 7a-d). The quantity of ^{14}C-GD internalised in organs was approximately 1.75% or 1% of the initially applied ^{14}C-GD for the untreated and Woundstat™ treated animals, respectively (Figure 8).

Discussion

The in vivo studies reported here used terminally anaesthetised large white pigs, with the GD challenge applied to the ear of the animal. This model and dosing site has been used previously for the assessment of countermeasures against nerve agents (17,18). In contrast to the previous studies, this is, to the authors' knowledge, the first time that the skin had been damaged to allow a more rapid ingress of chemical warfare agent, in order to model a non-haemorrhaging, contaminated wound.

The current study has shown that the use of WoundStat™ as a decontaminant on damaged (non-haemorrhaging) pig ear skin was unable to protect against GD toxicity. Most importantly, however, the use of WoundStat™ did not enhance the toxicity of GD.

WoundStat™ decontamination 30 seconds post GD challenge sequestered 68% ± 26% of the ^{14}C-GD applied. Contemporary studies evaluating WoundStat™ as a decontaminant against the vesicating agent sulphur mustard gave increased recoveries of 99% ± 8% (19). Given that similar experimental procedures were used, it is likely that the differences in recovery are ascribable to differences in physicochemical properties, such as volatility, between the two CWAs. In the case of GD, it is likely that a proportion volatilised or was absorbed prior to decontaminant application and was therefore not available for absorption by WoundStat™. Similar levels of WoundStat™ ^{14}C-GD decontamination were measured during *in vitro* studies for both damaged and undamaged skin (20), indicating that decontamination should be carried out as rapidly as possible to limit the percutaneous absorption of GD.

Decontamination with WoundStat™ substantially reduced the amount of ^{14}C-GD remaining on the skin surface (1.8% ± 1.9% versus 16.7% ± 20.8% for treated and untreated animals, respectively) and within the skin (7.5% ± 3.8% versus 42.7% ± 22.7%). The ^{14}C-GD remaining on or within the skin would not have impacted upon the observed and measured systemic toxicity. The systemic toxicity can be attributed to the internally absorbed dose of GD. WoundStat™ treatment resulted in a reduction of internally measured ^{14}C-GD by approximately 0.5% (1.2% ± 0.3%versus 1.7% ± 0.6%). This equated to a 30% reduction in systemically recovered material.

Despite this substantial reduction, whole blood cholinesterase measurements and ^{14}C-GD blood levels were similar whether or not WoundStat™ decontamination had been carried

out. Both experimental groups had animals that had high initial levels of ^{14}C-GD in the blood, associated with rapidly declining whole blood cholinesterase. These animals did not survive for more than 45 minutes into the exposure period. In the WoundStat™ decontaminated animals that survived the initial period, ^{14}C-GD blood levels and whole blood cholinesterase levels stabilised by 30 minutes and remained constant for the study duration. Conversely, for those animals in the untreated group that survived the initial period, cholinesterase levels remained below 5% of baseline values, whereas ^{14}C-GD blood levels increased steadily until death or study termination. For both the untreated and the WoundStat™ decontaminated animals, haematocrit levels increased steadily over the exposure duration, whereas for the control group haematocrit levels were consistently lower and remained constant. Importantly, although WoundStat™ was not an effective decontaminant against GD, it did not exacerbate GD toxicity. One of the major concerns regarding the treatment of GD-contaminated wounds was whether the use of a haemostat would increase the systemic absorption of GD. The current study found that WoundStat™ had no such effect.

In the current study, WoundStat™ was evaluated as a decontaminant in the absence of additional medical countermeasures. Should nerve agent exposure be confirmed, then further specific medical countermeasures would be employed alongside decontamination. The benefit of using decontaminants to increase survival times, allowing longer therapeutic windows for specific medical countermeasures to be employed, has been described previously (21).

Conclusion

The current study has shown that use of the haemostat WoundStat™, as a decontaminant for the nerve agent GD, was unable to protect against the effects of GD toxicity. Importantly, however, the findings of this study indicate that the use of WoundStat™ in GD-contaminated

wounds does not exacerbate GD toxicity.

Acknowledgements

Disclosure of interest

The authors report no conflicts of interest.

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Table 1. Observed signs of GD poisoning in the anaesthetised large white pig for the three experimental groups: Control (no GD, no treatment); Untreated (GD, no treatment); Treated (GD with WoundStat™ treatment 30 seconds post GD application). Each group contained 6 animals. 'Frequency' is the number of animals in the group that displayed the observable sign, with either the time to onset of the sign or the amount of saliva produced. Values are mean ± standard deviation calculated from the animals that responded.

		Control	Untreated	Treated
Miosis	Frequency	0	2*	4*
	Time to onset (min)	N/A	25 ± 18	71 ± 126
Mastication	Frequency	1	4	3
	Time to onset (min)	42	6 ± 1	7 ± 3
Fasciculation (head/face/neck)	Frequency	0	6	5
	Time to onset (min)	N/A	7 ± 9	11 ± 12
Fasciculation (limbs and rest of body)	Frequency	1	3	1
	Time to onset (min)	21	6 ± 1	13
Tremor / Convulsion	Frequency	0	3	0
	Time to onset (min)	N/A	33 ± 38	N/A
Hypersalivation	Frequency	1	5	4
	Amount (g)	3.84	19.4 ± 19.9	22.6 ± 18.4
Apnoea	Frequency	0	6[†]	4
	Time to onset (min)	N/A	80 ± 101	127 ± 153

*The incidence of miosis was lower in the untreated animals because 4 of them died too quickly for the observation/development of miosis.

[†]The animal in the untreated group that survived experienced a transient episode of apnoea.

Table 2. Monitored physiological signs in pigs during steady state anaesthesia both pre GD exposure and at the end of the study (immediately preceding apnoea or euthanasia) for the three experimental groups: Control (no GD, no treatment); Untreated (GD, no treatment); Treated (GD with WoundStat™ treatment 30 seconds post GD application). Values are mean ± standard deviation of n=6 animals. *Significant difference (p<0.05) between Pre-exposure and End values.

		Control	Untreated	Treated
Pulse rate	Pre-exposure	163 ± 19	163 ± 30	146 ± 34
	End	156 ± 31	115 ± 71	130 ± 24
Systolic pressure (mmHg)	Pre-exposure	120 ± 9	113 ± 7	122 ± 18
	End	106 ± 10	88 ± 36	108 ± 40
Diastolic pressure (mmHg)	Pre-exposure	93 ± 7	84 ± 7	93 ± 15
	End	79 ± 9	51 ± 36	70 ± 38
Mean pressure (mmHg)	Pre-exposure	107 ± 6*	99 ± 6	109 ± 18
	End	93 ± 8*	65 ± 38	87 ± 39
CO_2 (kPa)	Pre-exposure	6.2 ± 0.1	6.7 ± 0.6	7.2 ± 1.0
	End	5.9 ± 0.7	4.6 ± 3.7	7.5 ± 3.7
Breathing rate (min^{-1})	Pre-exposure	36 ± 13	29 ± 9*	33 ± 13*
	End	33 ± 9	6 ± 5*	17 ± 12*
SpO_2 (%)	Pre exposure	93 ± 3	91 ± 7*	88 ± 5
	End	92 ± 7	46 ± 40*	61 ± 36
Body temperature (°C)	Pre-exposure	38.2 ± 0.6	36.5 ± 2.2	36.7 ± 0.8*
	End	38.1 ± 0.5	36.6 ± 1.5	34.4 ± 2.2*

Figure legends

Figure 1. Survival curve for anaesthetised swine exposed to a multiple LD_{50} (300 µg kg^{-1}) dose of neat ^{14}C-GD via damaged ear skin (untreated; n=6); similarly exposed swine treated with the test decontaminant, WoundStat™ (treated; n=6); and anaesthetised unexposed animals (control; n=6).

Figure 2. Survival times for anaesthetised swine exposed to a multiple LD_{50} (300 µg kg^{-1}) dose of neat ^{14}C-GD via damaged ear skin (untreated; n=6); similarly exposed swine treated with the test decontaminant, WoundStat™ (treated; n=6); and anaesthetised unexposed animals (control; n=6). Individual data points are plotted and the central line represents the median value. The asterisk indicates a significant difference in survival time compared to the control group (capped bar; *p<0.05).

Figure 3. Total whole blood cholinesterase (ChE), shown as a percentage of the original (pre GD challenge) activity in anaesthetised pigs following damaged ear exposure to liquid GD. All values are mean ± standard deviation of up to 6 animals. All groups comprised 6 animals, with the untreated and treated animals receiving a 0.3 mg kg^{-1} GD challenge on to damaged ear skin. The treated animal group received WoundStat™ treatment at 30 seconds post GD contamination. The control group were not exposed to GD and did not receive treatment.

Figure 4. Total whole blood haematocrit (%) in anaesthetised pigs pre and post exposure to liquid GD on damaged ear skin. Pre exposure values were taken immediately prior to GD exposure, whilst the post exposure values were taken either immediately prior to the animal succumbing to GD toxicity, or upon completion of the 6 hour study duration. The treated animal group received WoundStat™ treatment at 30 seconds post GD contamination. The control group were not exposed to GD and did not receive treatment. All values are mean ± standard deviation of 6 animals. *Significant difference (p<0.05) between Pre- and Post-exposure values.

Figure 5. Amount of ^{14}C-GD present in the blood of large white pigs after GD application on to damaged ear skin for untreated and WoundStat™-treated animals. One animal in the untreated group and two animals in the treated group survived for the maximum study duration of 6 hours. Those animals with larger amounts of ^{14}C-GD present in the blood earlier in the time course succumbed to the toxic effects of GD within 45 minutes of challenge. Each animal received a GD dose at t=0 of 0.3 mg kg^{-1}. All pre GD challenge values are mean ± standard deviation of n=6 animals, which decreased as animals succumbed to GD toxicity over the study duration.

Figure 6. Comparison of whole blood cholinesterase (% of original) and the amount of ^{14}C-GD (or breakdown products) in the blood (determined from quantification of radiolabel) for untreated and WoundStat™-treated animals. Each animal received a GD dose of 0.3 mg kg^{-1} on to damaged ear skin. A least-squares fit non-linear regression analysis was used to curve fit the data for each group. The equations for the curves were: Untreated Y= (92.22-1.484)*exp(-0.2739X) + 1.484 and Treated Y= (95.42-3.604)*exp(-0.1930X) + 3.604.

Figure 7. ^{14}C-GD distribution for WoundStat™-treated and untreated animals after a 0.3 mg kg^{-1} damaged ear skin exposure, expressed as a percentage of the applied dose.

A. Total Recovery. Sampled compartments were: WoundStat™-sequestered (treated animals only), GD remaining on damaged skin surface, GD present with the skin directly under the dosing site, GD present within the skin adjacent to the dosing site, GD absorbed into the dosing assembly and GD recovered from sampled organs (detailed in Figure 6). Compartments were sampled either after the animal had succumbed to GD toxicity or upon completion of the 6 hour study duration.

B. External Quantification. Compartments shown: WoundStat™-sequestered (treated animals only), GD remaining on damaged skin surface and GD absorbed into the dosing assembly.

C. Local Quantification. Compartments shown: GD present with the skin directly under the dosing site and GD present within the skin adjacent to the dosing site.

D. Internal Quantification. Compartment shown: GD recovered from sampled organs (detailed in Figure 6).

All values are mean ± standard deviation of n=6 animals. *†Statistically significant (p<0.05) differences in the respective parameters between the Treated and Untreated groups.

Figure 8. ^{14}C-GD internal organ distribution for WoundStat™-treated and untreated animals after a 0.3 mg kg^{-1} damaged ear skin exposure, expressed as a percentage of the applied dose. Sampled internal organs were heart, spleen, lung, pancreas, liver, kidney, blood and brain. Organs were harvested after either the animal had succumbed to GD toxicity or upon completion of the 6 hour study duration. Recovery from within the systemic circulation was determined from the final blood sample taken prior to the end of each study. All values are mean ± standard deviation of n=6 animals. *Statistically significant (p<0.05) difference between the Treated and Untreated groups.