J. Pharm. Pharmacol. 1980, 32: 757–760 Received April 24, 1980

Influence of the sterilization method and of magnesium oxide on the tissue responses in the rat to modified starch glove powders

D. PELLING* AND K. R. BUTTERWORTH

British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, SM5 4DS, U.K.

The tissue response to samples of a surgical glove powder was assessed from counts of the adhesions and granulomas found 7 and 14 days after introduction into the peritoneal cavity of rats, and by a histological study of affected tissues. Negative control groups treated with no powder and positive controls treated with talc were included. Various sample treatments were implemented to examine the effect of the sterilization method on the tissue response and the influence of magnesium oxide, normally added as a dispersing agent. The glove powder produced less reaction when steam-sterilized than when γ-irradiated and the presence of magnesium oxide at 2% concentration made no detectable difference.

First observations on the use of modified starch powders as substitutes for talc for dusting surgical gloves showed promise (Lee & Lehman 1947; MacQuiddy & Tollman 1948), but it soon became apparent that starch powder could persist unabsorbed in the body for several weeks and produce untoward effects (Myers et al 1960; Myllärniemi et al 1966). However, starch glove powders continue to be used and cases of wound contamination and post-operative complications continue to be reported (Aarons & Fitzgerald 1974).

The present study was carried out on a glove powder in common use and consisting of corn starch modified chemically and physically to inhibit aggregation of the particles during sterilization (Bio-Sorb). It contains 2% light magnesium oxide to improve its flow properties. Since any of the preparative steps might modify the tissue reaction evoked, two experiments were carried out with the aims of identifying conditions for sterilization which result in the least reactive preparation, and of examining the influence of magnesium oxide on the tissue response.

MATERIALS AND METHODS

Materials

The materials tested and sterilization procedures used are listed in Tables 1 and 2. Each experiment included a negative control group given a sham operation and a positive control treated with talc. The product samples sterilized by steam had 'caked' and were restored to a powder by gentle crushing before use. Sterilization of the dry powder samples was carried out on 1·5 g samples in the 4 × 5·5 cm paper sachets normally packed with surgeons' gloves.

Methods

Polystyrene scoops were prepared from truncated LP2 test tubes (Luckham Ltd., Sussex) fixed to wire handles and calibrated to hold 50 mg powder per levelled measure. One scoop was used for each sample after exposure to u.v. irradiation for 24 h. The mean weight of six samples of powder measured in each scoop varied between 47·6 and 52·3 mg and the standard deviation of each mean between 0·8 and 3·3 mg. The densities of talc and light magnesium oxide were about one third and one seventh those of the powder, therefore one measure delivered 17·4 and 7·7 mg respectively.

Each sample was tested in a group of 16 rats, 8 of which were examined after 7 days, and 8 after 14 days. The samples and animal cages were code-labelled to prevent identification of the treatments by the operator. Female Wistar rats, 125–225 g were randomly assigned to their groups and anaesthetized with ether. The abdomen was opened by mid-line incision using aseptic technique and one measure of the sample sprinkled over the abdominal viscera. The body wall and the skin incision were each closed with a continuous silk suture. The powder sample in aqueous suspension was applied by pipette in a volume calculated to contain 50 mg. After 7 or 14 days the animals were killed with chloroform. The abdominal viscera were examined and records made of the numbers, sizes and locations of any lesions. An upper limit of 30 was made on the number of granulomas counted in any one animal. The four animals having the most lesions in each

* Correspondence.

group were selected for histopathological examination. Samples of all the tissues with visible lesions in these animals were fixed in 10% buffered formalin, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin, and examined microscopically.

RESULTS

Post-mortem examination

By far the most common lesions were small granulomas 0·5–2·0 mm diameter on the peritoneum and adhesions between organs, or between organs and the body wall. The frequencies of these lesions were used as a basis for comparing the reaction of the tissues to the samples.

The susceptibility of the various abdominal organs to the powder-induced lesions is indicated by the following figures for the overall frequencies of involvement of the organs with each type of lesion. Experiment 1—Adhesions: body wall 46; omentum 35; liver 26; ileum 21; pancreas 17; caecum 14; stomach 12; bladder 7; ovary 3; uterus 3; colon 1. Granulomas: body wall 47; liver 17; caecum 4; ileum 3; pancreas, stomach and omentum 2 and duodenum 1.

Incidence of adhesions and granulomas

The mean incidences of the lesions are shown in Tables 1 and 2. Differences between all possible pairs of treatments were examined in each experiment using the method of Wilcoxon & Wilcox (1964).

In general, the numbers of adhesions produced by each sample were similar at 7 and 14 days but there was a tendency towards fewer granulomas after the longer period.

All the powder samples produced markedly fewer granulomas than talc, although with the statistical test used the differences were not significant in every case.

In experiment 1 (Table 1) all three steam-sterilized powder samples produced significantly fewer granulomas than talc after 7 and 14 days, whereas the numbers after γ-irradiation or dry heat sterilization were not significantly less than with talc. One powder sample sterilized by γ-irradiation (recovered after a glove coating cycle) produced significantly more adhesions than were found in the sham-operated groups and in a group treated with the steam-sterilized powder.

In experiment 2 (Table 2) there was no evidence of a difference between the powder samples containing and those not containing 2% light magnesium oxide. However, both the samples of light magnesium oxide produced significantly more lesions than the sham-operated controls, and in two instances more granulomas than steam-sterilized powder samples (legend to Table 2). The steam-sterilized powder samples almost invariably produced significantly fewer lesions than the talc-positive controls and this finding is consistent with the results of experiment 1. The γ-irradiated samples, however, held an intermediate position in both experiments, with few significant differences from either the sham-operated or talc controls.

Table 1. Frequencies of adhesions and granulomas in rats exposed to intraperitoneal application of glove powder sterilized by different methods—experiment 1.

	Adhesions		Granulomas	
Treatment group	7 days	14 days	7 days	14 days
Sham-operated control	0·17	0	0**	0**
Standard powder (γ)	0·75	1·13	2·63	0·88
Standard powder recycled[a] (γ)	2·50††	2·25†	1·63	0·50**
Standard powder 60 min 160 °C (dry heat)	1·13	1·13	4·50	1·13
Standard powder 3 min 134 °C (steam)	0·63	0·25	0·25**	0·75*
Standard powder[b] 3 min 134 °C (steam)	0·75	0·88	0·25**	0·75**
Standard powder 20 min 121 °C (steam)	0·38	0·63	1·25*	0·75*
Talc B.P. 20 min 121 °C (steam)	1·83†	0·50	30††	24††

[a]: subjected to normal glove coating cycle, dried by heating to 70 °C recovered and sterilized by γ-irradiation. This sample also produced more adhesions at 7 days than the powder sterilized at 20 min 121 °C steam ($P < 0·01$).

[b]: sterilized in aqueous suspension.

γ: γ-irradiated 2·5 Mrad.

Values are means for groups of 8 animals.

Intergroup differences were examined by a non-parametric test (Wilcoxon & Wilcox 1964). Values significantly less than those of the talc positive control: $*P < 0·05$; $**P < 0·01$.

Values significantly greater than those of the sham-operated control: $\dagger P < 0·05$; $\dagger\dagger P < 0·01$.

Histopathology

Microscopic examination of the tissue sections between crossed polarizing filters revealed starch granules (Fig. 1) or talc particles associated with the granulomas and adhesions. Light magnesium oxide is not birefringent but is basophilic and could be recognized as an amorphous granular mass or masses staining mauve with haematoxylin and

Table 2. Frequencies of adhesions and granulomas in rats exposed to intraperitoneal application of glove powder, with and without magnesium oxide and sterilized by different methods—experiment 2.

	Adhesions		Granulomas	
Treatment group	7 days	14 days	7 days	14 days
Sham-operated control	0·88*	0·50**	0·13**	0**
Standard powder (γ)	1·75	0·88	1·50*	3·00
Powder without MgO (γ)	1·13*	1·25	2·13	1·25**
MgO-(γ)	1·25	1·00	8·88†	8·75†
Standard powder (steam)	1·13*	1·00	0·88**	0·13**
Powder without MgO (steam)	0·38**	0·17*	0·50**	0·57**
MgO (steam)	1·63*	2·63†	17·0††	9·75††
Talc[a] (γ)	5·00††	3·25††	30††	30††

Values are means for groups of 8 animals.
[a]: 5 μm Stearic 'O' talc supplied by Luzenac, France.
MgO: light magnesium oxide B.P.
γ: γ-irradiated 2·5 Mrad.
steam: autoclaved 3 min 134 °C.
Intergroup differences were examined by a non-parametric test (Wilcoxon & Wilcox 1964). Values significantly less than those of the talc positive control: *$P < 0{\cdot}05$; **$P < 0{\cdot}01$.
Values significantly greater than those of the sham-operated control: †$P < 0{\cdot}05$; ††$P < 0{\cdot}01$. In addition to the differences indicated, MgO (steam) produced more granulomas at 7 days than powder without MgO (steam) ($P < 0{\cdot}05$), and MgO γ produced more granulomas at 14 days than standard powder (steam) ($P < 0{\cdot}05$).

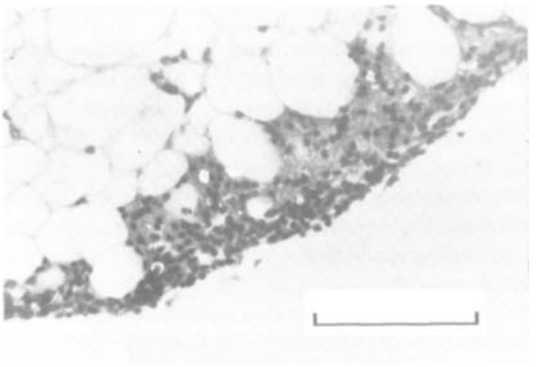

FIG. 1. Small granuloma on the surface of adipose tissue taken from a rat 14 days after treatment with the powder. Section stained with haematoxylin and eosin, and photographed between partially crossed polarizing filters. The starch granules appear as 'maltese crosses' or rings. Scale = 100 μm.

eosin. Granulomatous tissue surrounded each mass, with giant cell, mast cell and inflammatory cell infiltration. Starch granules were either distributed throughout the granuloma or concentrated at the periphery, but there was no evidence of dense aggregations of starch.

Material of the type found in the animals treated with magnesium oxide alone were not seen in those treated with the powder containing 2% magnesium oxide. Also, there were no consistent differences in the appearance of the granulomas produced by the powder with or without magnesium oxide.

DISCUSSION

A non-parametric test of statistical significance was used to compare the lesion frequencies because the response data were not distributed normally. The method may be regarded as conservative because the efficiency of the ranking method was lessened by the high proportion of tied ranks. Few comparisons between pairs of test treatments attained statistical significance and these are given in the legends to the Tables. Instead, the statistical criteria for comparing the tissue reactions from the samples relies chiefly on the differences in lesion frequencies between the test and the two control treatments. Consistently fewer lesions were counted in animals exposed to steam-sterilized powder than to γ-irradiated powder in both experiments, and this is borne out by the statistical treatment used. A more rigorous statistical test (Student's-t) was applied after transformation of the granuloma count data by the method of Freeman & Tukey (1950). This indicated that fewer granulomas were produced by the steam-sterilized powder compared with γ-irradiated powder after 14 days in experiment 2 ($P < 0{\cdot}01$) but that the difference after 7 days was not statistically significant. Steam sterilization altered the physical characteristics of the powder and light crushing was necessary to restore the 'caked' material to a powder. Although smaller unbroken aggregates may have survived to be introduced into the animals, this might be expected to retard rather than hasten the absorption of the steam-sterilized samples.

The histological study showed starch granules remaining in the tissues for at least 14 days. The trend towards fewer granulomas after 14 days than after 7 days probably reflects the process of starch absorption. These findings support those of Myers et al (1960) in rabbits, in which the number of granulomas began to fall after one week but resolution of the adhesions took longer.

The commercial preparation contains 2% light magnesium oxide to improve its flow characteristics. In the present study, light magnesium oxide has been

shown to induce a well-defined lesion on its own, hence raising questions about the relative importance of light magnesium oxide in producing the so-called starch granuloma. However, in the second experiment, no differences were detectable between the samples containing light magnesium oxide and those not containing it, irrespective of the sterilization method. The importance of a dispersal agent such as magnesium oxide in promoting absorption has been emphasised by Lee et al (1952). Myllärniemi et al (1966) investigated the role of magnesium oxide in the formation of abdominal adhesions and granulomas in rabbits. In their study, magnesium oxide administered alone produced fewer lesions than Bio-Sorb. This difference from our results could be due to the different animal species or to their longer periods of study (1, 2 and 3 months). Graham & Jenkins (1952) found that the peritoneal response to magnesium oxide and Bio-Sorb were similar when the same weights were applied. These papers do not permit a conclusion about the influence of magnesium oxide at 2% concentration in the powder, because neither included a control group using the powder without the dispersal agent. Our experiments suggest that the irritancy contributed by light magnesium oxide, or any advantage it confers by facilitating dispersal of the Bio-Sorb, may be of no practical importance and in any case produces smaller differences than may be attributed to the method of sterilization.

Using peritoneal adhesion and granuloma counts as the criteria for tissue response it is concluded that the powder was less irritant when sterilized by steam than when γ-irradiated. The presence of light magnesium oxide at 2% concentration made no significant difference to the incidence of the lesions.

Acknowledgements

The authors wish to thank London Rubber Company, Products Ltd., for their financial support and Mr D. Bratby of L.R.C. for supplying the test materials.

REFERENCES

Aarons, I., Fitzgerald, N. (1974) Surg. Gynecol. Obstet. 138: 385–390

Freeman & Tukey (1950) Ann. Math. Stat. 21: 607 as cited Documenta Geigy. 6th edn Geigy Pharmaceutical Co. Ltd. Manchester, p 186

Graham, J. D. P., Jenkins, M. E. (1952) J. Pharm. Pharmacol. 4: 392–398

Lee, C. M., Collins, W. T., Largen, T. L. (1952) Surg. Gynecol. Obstet. 95: 725–737

Lee, C. M., Lehman, E. P. (1947) Ibid. 84: 689–695

MacQuiddy, E. L., Tollman, J. P. (1948) Surgery 23: 786–793

Myers, R. N., Deaver, J. M., Brown, C. E. (1960) Ann. Surg. 151: 106–112

Myllärniemi, H., Frilander, M., Turunen, M., Saxen, L. (1966) Acta Chir. Scand. 131: 312–318

Wilcoxon, F., Wilcox, R. A. (1964) Some Rapid Approximate Statistical Procedures. Lederle Laboratories, New York, pp 9–11