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DEVELOPMENT OF LOCAL MORPHOLOGICAL CHANGES AFTER INTRADERMAL INOCULATION OF Q-FEVER CHEMOVACCINE

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Summary. — Development of morphological changes was studied in the site of intradermal inoculation of Q-fever chemovaccine. The chemovaccine was obtained by trichloroacetic acid extraction from *Coxiella burnetii*, strain Nine mile, phase I cells. The effect of two vaccine doses (0.2 mg and 1.0 mg) was compared in two groups of guinea pigs (each consisting of 21 animals). After inoculation of 1.0 mg vaccine abscesses formed in the dermis and in subcutaneous tissue along with dystrophic changes of skeletal muscles. Reparation of these lesions progressed sufficiently slowly and was not completed before 60 days after vaccine inoculation. The regeneration of skeletal muscles was of budding type. After inoculation of 0.2 mg of chemovaccine minimal lesions occured in the subcutaneous tissue only. However, already within 48 hr this vaccine dose evoked a strong reparation response. Interesting was the finding of Kurloff cells in the haemorrhagic foci. The results suggest that the low-reactogenic chemovaccine dose of 0.2 mg might be sufficiently immunogenic after subcutaneous administration.

Key words: *Q-fever*; *chemovaccine*; *guinea pig*; *morphology*; *skin reaction*; *Kurloff cells*

Introduction

Subcutaneous administration of substances with therapeutic and/or prophylactic effect is used when the preparation is not resorbed from gastrointestinal tract or when it is degradated by digestive enzymes, in order to achieve quickly a high concentration in blood or for elimination of side effects coupled with other routes of application. However, subcutaneous or intramuscular administration of biologically active substances is accompanied with side effects — mainly local damage of tissues (Rešl and Kuna, 1984, 1985; Balogh, 1986). In particular, subcutaneous application of the substance in medical practice depends on the degree of its local damaging effect.

Application for man of the chemovaccine against Q-fever prepared in the Institute of Virology, Slovak Academy of Sciences, Bratislava (Brezina and

Úrvölgyi, 1962) showed that after subcutaneous inoculation a local reaction developed characterized by hyperhaemia and infiltration which in some cases lasted for a sufficiently long time (Kazár *et al.*, 1982, 1983). The histology of this local reaction was not studied. For a more common use of the chemovaccine in medical practice, the character of local postvaccination reactions must be studied in more details. The aim of this work was to follow the dynamics of local changes in guinea pigs which were exposed to different doses of the preparation by subcutaneous route.

Materials and Methods

Animals. Guinea pigs weighing 250—300 g, 5—7 weeks old, were obtained from the farm VELAZ (Prague). The animals were housed under stabile environmental conditions and allowed free access to standard diet KO-16 (VELAZ, Prague).

Vaccine. Chemovaccine against Q-fever was prepared in the Institute of Virology, Bratislava as described (Brezina and Úrvölgyi, 1962). The lot No. 16, expedition date 17. 2. 1986 was used. 1.0 mg of the preparation contains the trichloroacetic acid extract from 1.0 mg of purified *Coxiella burnetii* Nine Mile strain in phase I. Vaccine was applied subcutaneously into the right hind thigh. We used two doses: 1.0 ml of undiluted vaccine (extract from 1.0 mg of *C. burnetii*); 1.0 ml of vaccine diluted 1 : 5 (extract from 0.2 mg of *C. burnetii*).

Experimental design. One group containing 21 guinea pigs was used to study the skin reactions or each vaccine dose. Samples for morphological examination were obtained 12, 24, 48 hr and 6, 12, 30 and 60 days after vaccine application, respectively (3 guinea pigs for each time interval). Animals were killed by ether, the tissues were removed from the inoculation site. Paraffin sections 7 μm thick were prepared using standard histological procedures; they were stained with haematoxylin-eosin and according to van Gieson.

Results

By 12 hr after vaccination with the 1.0 mg dose haemorrhages were seen in dermal and subcutaneous tissue accompanied with a mild infiltration of neutrophil granulocytes. After administration of 0.2 mg dose the extent of haemorrhages was markedly lower, however, the granulocyte infiltration was even more pronounced. No changes in skeletal muscles were observed at this interval after either vaccination doses.

By 24 hr after vaccine application in the 1.0 mg dose haemorrhages were found not only in dermal and subcutaneous tissues, but also in the underlying skeletal muscles. Swelling of intercellular connective tissue, oedema and granular dystrophy of muscle fibres were associated with leukocyte infiltration (Fig. 1). After administration of the 0.2 mg dose infiltration of the regions of haemorrhages continued, but skeletal muscles showed no changes.

At 48 hr after vaccine inoculation in the 1.0 mg dose, the extent of lesions in muscle tissue increased; in the dermis and subcutaneous connective tissue abscesses developed. After 0.2 mg dose granulocyte infiltration in the subcutaneous tissue has continued, histiocytes appeared but no abscesses formed and the muscle fibres were not damaged.

By 6 days after application of vaccine in the 1.0 mg dose, foci of reparation of the skeletal muscle of budding type were found, e.i. the formation of new fibres which in transversal sections showed round shaped, of different size with basophilic sarcoplasm (Fig. 2). Newly formed fibres substituted the defects

in the muscle tissue; this was infiltrated by abundant histiocytic mononuclear cells. Subcutaneous tissue exhibited foci of abscess formation. After 0.2 mg dose the defects in hypodermal tissue were replaced by cellular connective tissue.

By 12 days after vaccination with the 1.0 mg dose in all cases formation of encapsulated abscess was found, penetrating from epidermis through the dermis to subcutaneous tissue and reaching the upper margin of skeletal muscle. In the foci of haemorrhages Kurloff cells were seen. After the 0.2 mg dose in comparison with the 6 days interval, intensive vascularized fresh connective tissue was observed, in which fibrin deposits were detected as aemorrhage remnants.

By 30 days after vaccine application in the 1.0 mg dose defects of dermal and subcutaneous tissue in the site of abscesses were substituted with young connective tissue. After the 0.2 mg dose decreased quantity of cellular elements in young connective tissue was seen with progressed sclerotization nd scar formation.

By 60 days after vaccine injection in the 1.0 mg dose at the place of vaccine application normal dermal tissue was substituted with connective tissue. Epidermis was unchanged. After application of the 0.2 mg vaccine dose no pathological changes were observed.

Discussion

The results document that the chemovaccine against Q-fever possesses a marked damaging activity on guinea pig tissues. In these animals, the dose of 1.0 mg vaccine (equal to 1 dose for man according to recommendation of authors) provoked strong morphological changes at the place of their application. During 48 hr after injection advanced suppurative interstitial inflammation was found in the subcutaneous tissue. Reparation of these changes continued relatively slowly so that it was not completed 60 days after vaccination. Characteristically, lesions were detected not only in the vicinity of inoculation site in subcutaneous tissue, but also in underlying skeletal muscle where dystrophic changes (granular dystrophy of sarcoplasm) were seen with transition to focal necrosis. The defect reparation was in part of budding type, in part substitution with connective tissue was observed as a consequence of productive inflammation.

Kurloff cells are a very interesting finding in the foci of haemorrhages (after injection of the 1.0 mg vaccine dose) (Sandberg and Hagelin, 1986). These cells were repeatedly seen in the spleen of immunized guinea pigs; their quantity showed a marked dynamics related to the postvaccination interval (unpublished observations). So far we found no reports on increase of the number of Kurloff cells as result of chemovaccine application. Appearance of these cells in the foci of haemorrhages can be considered as a reflection of their quantity in peripheral blood.

Subcutaneous injection of the chemovaccine in the 0.2 mg dose resulted in a more marked leucocytic reaction (exudative inflammation) in comparison with the dose of 1.0 mg. This finding documents the prevalence of

reparatory processes above alteration. Skeletal muscles were not damaged after application of vaccine in this dose. An important criterion for characterization of the preparation is its high protective and immunogenic effect in the given dose at experimental Q-fever infection of guinea pigs (Votruba *et al.*, 1985; Kazár *et al.*, 1986).

It is noteworthy that the use of both doses of vaccine led to lesions in subcutaneous tissue together with concomitant haemorrhage. Disturbances of haemocoagulation and trombocytopoesis were described after infection of animals with live *Coxiella* as after application of dead cells (Jakubovský *et al.*, 1985). From these data arises the hypothesis that the haemorrhage in the site of chemovaccine injection resulted from specific action of *Coxiella* components contained in the preparation (probably lipopolysaccharides in nature) on the tissues of animals. However, the haemorrhage in the inoculation site may also be a consequence of mechanical tissue damage (Rešl and Kuna, 1985).

The local reaction after subcutaneous injection of the chemovaccine in guinea pigs exhibited the feature of exudative or suppurative inflammation depending on the vaccine dose. Exudative inflammation after inoculation of an immunologically effective dose (0.2 mg) had a favourable course; reparative changes proceeded during 30—60 days after vaccination without side effects.

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Legend to Figures (Plates XXIII—XXIV):

Fig. 1. Granular dystrophy of skeletal muscle sarcoplasm in transversal section (arrows). Magnification × 120, haematoxylin-eosin.

Fig. 2. Focus of regeneration of skeletal muscle (arrows). Magnification × 250, haematoxylin-eosin.