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Olive oil: suitability for use as a vehicle in the local lymph node assay

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Many methods for the identification of skin sensitization potential have been described (1-3), none without its difficulties, including the choice of vehicle (4, 5). The local lymph node assay (LLNA) (6-8) has the advantage that it uses only topical application of test chemical, but this in turn makes the choice of vehicle particularly important (7). The vehicle of choice suggested has been acetone: olive oil, 4:1, v/v. However, the use of olive oil has been criticized on the basis that it may itself cause contact allergy (9) and may elicit proliferative responses in the LLNA (10). In this paper, we report our experience with olive oil in the LLNA.

Materials and Method

Materials

Olive oil was purchased from Sigma Chemical Co, Poole, Dorset, UK. It was aged by storage at ambient tempera-

ture, under normal laboratory lighting conditions, for 6 months. ³H-methyl thymidine (product code TRA.310, specific activity 2 Ci/mMol concentration 1 mCi/ml) was obtained from Amersham International, Amersham, Bucks, UK.

Local lymph node assay

Local lymph node assays were performed according to the standard protocol (7). CBA/Ca strain mice (6-8 weeks; Harlan Olac, Bicester, UK) were used throughout. 25 µl of the test material or the vehicle alone were applied to the dorsum of both ears of groups of 4 mice. Treatment was performed 1× daily for 3 days, 5 days following the initiation of exposure, all mice were injected via the tail vein with 250 µl of phosphate-buffered saline (PBS) containing 20 µCi of ³H-methyl thymidine (³HTdR). Mice were sacrificed 5 h later and the draining auricular lymph nodes excised and pooled for each experimental group. A single cell suspension of lymph node cells (LNC) was prepared by gentle mechanical disaggregation through 200-mesh stainless steel gauze. Pooled LNC were washed 2× with an excess of PBS and precipitated with 5% trichloroacetic acid (TCA) at 4°C for 18 h. Pellets were resuspended in 1 ml TCA and transferred to 10 ml of scintillation fluid (Optiphase MP;

Table 1. Sensitization potential of fresh and aged olive oil in the LLNA

Treatment regime	Fresh olive oil		Olive oil aged for 6 months	
	dpm/node ^{a)}	T/C ratio ^{b)}	dpm/node	T/C ratio
none	306	—	335	—
100% acetone	152	0.5	304	0.9
80:20 AOO	321	1.0	490	1.5
50:50 AOO	661	2.2	478	1.4
20:80 AOO	708	2.3	934	2.8
100% olive oil	966	3.2	1192	3.6

^{a)} Disintegrations per min per lymph node.

^{b)} Ratio of test to control dpm/node.

Table 2. Historic values for proliferation in untreated and AOO-treated draining lymph nodes

	Range, mean dpm/node ± standard deviation
untreated controls	306-576, 394 ± 107
AOO-treated controls	223-850, 504 ± 139

LKB). Incorporation of $^3\text{HTdR}$ was measured by β -scintillation counting as disintegrations per min (dpm) for each experimental group.

Results

The results of local lymph node assays conducted with fresh olive oil and olive oil stored for 6 months are presented in Table 1. In each case, there was a low-level stimulation of lymph-node cell proliferative activity, with some evidence for a dose-response relationship. As the ratio of test to control values exceeds 3, the results obtained with both fresh and aged undiluted olive oil would be regarded as indicative of skin sensitization potential (7). However, it is clear that there was no real difference between the 2 samples. As the recommended 1st choice vehicle for the LLNA is a ratio of 4 parts acetone to 1 part olive oil (i.e., a 20% solution of olive oil in acetone), analyses were performed with this mixture (4:1 AOO). The results obtained with the aged olive oil were slightly higher than those recorded using the fresh material. Nevertheless, these values were well within those observed during the course of a number of previous experiments, where the dpm/node values for naive controls were 394 ± 107 ($n=4$) and the dpm/node values for 4:1 AOO treated controls were 504 ± 139 ($n=50$).

Discussion

Montelius et al. (10) have reported evidence of more substantial proliferative responses to undiluted olive oil, with dpm/node values in the range of 2261–5839. Such a strong response is surprising in view of the weak sensitization potential of olive oil, which is only very rarely implicated in allergic contact dermatitis, and then usually in association with venous ulcers (9). For 4:1 acetone:olive oil, their values were in the range of 213–1160 dpm/node (10). These latter results are close to our experience reported here of 223–850 dpm/node, this having been collated from some 50 studies as representative of the more than 200 sets of data available. Thus, it has been our experience that a 4:1 v/v mixture of acetone and olive oil offers the desired properties of a suitable vehicle and extensive datasets have been generated using it (8, 11, 12). The data reported here further support the use of this vehicle, though there is no doubt that other vehicles should be used when they are more appropriate.

A list of alternatives has been suggested (in order of preference, dimethyl formamide, methyl ethyl ketone, propylene glycol and dimethyl sulphoxide) (7), but there seems to be good evidence that 4:1 AOO is a reasonable first-choice vehicle.

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