The effect of 4-undecanone and its derivatives on cellular and humoral immunity and tumor growth in mice

JULIA GIBKA[1], EWA SKOPIŃSKA-RÓŻEWSKA[2], ALEKSANDER WASIUTYŃSKI[2], EWA SOMMER[2], ANDRZEJ K. SIWICKI[3], JANUSZ BANY[4], HENRYK SKURZAK[5], MICHAŁ MAZURKIEWICZ[2]

[1]Institute of General Food Chemistry, Technical University of Łódź; [2]Department of Pathology, Biostructure Center, Warsaw Medical University; [3] Department of Microbiology and Clinical Immunology, Warmian-Mazurian University, Olsztyn; [4]Department of Toxicology and Pharmacology, Military Institute of Hygiene and Epidemiology, Warsaw; [5]Department of Immunology, Cancer Center, Warsaw, Poland

Abstract

4-undecanone (4-on) was used as the starting material in the synthesis of undecan-4-ol (4-ol) and 4-undecanone ethylene and propylene acetals (Acet4 and Acpr4). The in vivo effects of these substances on cellular and humoral immunity and on the syngeneic Sarcoma L-1 tumor growth were studied in inbred Balb/c mice and BDF1 hybrids. Mice were subjected to inhalation for three days. An increase of antibody production in mice which inhaled 10% 4-ol, 1% Acet4 or 1% Acpr4 were observed. The effect of 4-ol on cellular immunity(local graft-versus host reaction)depended on the scheme of experiment: was stimulatory when BDF1 recipients mice were inhaled after Balb/c donors lymphocytes grafting, and inhibitory when recipients obtained cells from inhaled parental Balb/c mice. However, when BDF1 hybrids were grafted with lymphocytes obtained from inhaled BDF1 donors, stimulatory effect was observed. No effects of inhaled 4-on were observed.. Intracutaneusly grafted Sarcoma L-1 tumors were significantly smaller in mice inhaled 4-ol, Acet4 and Acpr4 than in mice inhaled 4-on as well as those from the control group.

Key words: *4-undecanone, mice, cellular immunity, humoral immunity, syngeneic tumor.*

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Introduction

A smell is information for the immune and nervous system to change their level of activity. The signals from nerve cells expressing the same odorant receptor are transmitted to second-order neurons, located in the olfactory bulb and then to the olfactory cortex. Immune cells receive these signals via receptors for neurotransmitters and hormones [1-10]. Aliphatic methyl ketones, products of metabolic transformation of fatty acids, are widely distributed in nature. They are present, among others, in odorous secretions of insects, essential oils of various plants, fruits infected with fungi [11-18]. 2-undecanone and some of its derivatives are in the list of artificial flavouring substances that may be added to fragrances and food without hazard to public health.

Recently, we have reported the immunotropic effects of 2-undecanone (2-on), 3-undecanone (3-on) and their derivatives [19-21].

4-undecanone and its derivatives, until now, have not been found in the nature.

The aim of the present study was to evaluate the effect of 4-undecanone (4-on) and its derivatives: undecan-4-ol (4-ol) and ethylene and propylene acetals (Acet4 and Acpr4) on cellular and humoral immunity and on the growth of syngeneic tumor in mice.

Materials and Methods

Materials

4-undecanone and its derivatives: undecan-4-ol and both acetals, were synthesized by dr Julia Gibka (Technical

Correspondence: Ewa Skopińska-Różewska, Pathology Department, Biostructure Center, Warsaw Medical University, Chałubińskiego 5, 02-004 Warsaw, Poland. Email: ewaskop@hotmail.com

University of Łódź) and dr Marek Gliński (Technical University of Warsaw)as described previously [22, 23]. A correlation between the structure of the studied compounds and their odors has been found. The shift of the functional group to the position 4 for the ethylene and propylene acetals strengthened their vegetable and spicy odors.

Mice

The study was performed on 10-12 week old inbred Balb/c mice, weighing 25-28 g, of both sexes, delivered from a breeding colony of the Polish Academy of Sciences and Warsaw University, and on F1 hybrids Balb/c x DBA2 (BDF1) from own breeding colony.

Inhalations

Mice were subjected to inhalation for 3 days, according to the following scheme: 5 mice in one cage, 5 drops of tested substance for 60 minutes, cage covered by linen during inhalation. Cages with control mice were accordingly covered by linen for 60 minutes.

A part of donor mice was subjected to inhalations before taking material (spleen lymphocytes) for performing local graft-versus-host test. Other animals (recipients of splenic lymphocytes or sheep red blood cells) were subjected to inhalations after introducing these cells.

Study of antibodies production [24]

F1 (Balb/c x DBA/2)12 weeks old female mice were immunized with 10% SRBC (0.1 ml intraperitoneally), inhaled in days 0, +1 and +2 and bled 7 days after immunization. Antibody level was evaluated with haemagglutination assay in inactivated (56°C, 30 min) sera. After performing serial sera dilutions, 0.5% SRBC were added and the mixture was incubated for 60 min at room temperature, then centrifuged (10', 150 g) and shaked. Hemagglutination titer was evaluated in light microscope – as a last dilution where at least 3 cells conglomerates were present in at least 3 consecutive fields at objective magnification 20x.

Local graft-versus-host reaction (immunological angiogenesis, LIA test)

Local GvH reaction (lymphocyte- induced angiogenesis test, LIA) was performed according to Sidky & Auerbach [25] with some modifications [19]. Briefly, 12 weeks old female Balb/c mice were sacrificed with Morbital, spleens were dissected and spleen cells suspensions were grafted intradermally (500 thousands of cells in 0.05 ml of Parker medium per graft) into female 10 weeks old (Balb/c x DBA2) F1 mice. Before performing injections mice were anaesthetized with 3.6% chloralhydrate (0.1ml per 10 g of body weight). Both flanks of each mouse were finely shaved with razor blade, on each flank we localized 2-3 injections. Cell suspensions were supplemented with 0.05 ml/ml of 0.01% trypan blue in order to facilitate recognition of injection sites later on.

Grafted Balb/c spleen cells recognized DBA/2 antigens and produced many immunological mediators including proangiogenic factors (immunological angiogenesis). In this case number of newly formed blood vessels was the measure of cells reactivity. After 72 hours mice were treated with lethal dose of Morbital. All newly formed blood vessels were identified and counted in dissection microscope on the inner skin surface, using criteria suggested by the authors of the method, at magnification of 6x, in 1/3 central area of microscopic field. Identification was based on the fact that new blood vessels, directed to the point of cells injection, differ from the background vasculature in their tortuosity and divarications.

Evaluation of sarcoma L1 development after tumor cells grafting in mice

Sarcoma cells were delivered from the Warsaw's Cancer Center collection and then passed through three generations of male Balb/c mice [26]. Multiple 0.05 ml samples of 200 thousands of cells were injected intradermally into partly shaved narcotised 10-12 weeks old male Balb/c mice. These mice were then subjected to inhalation with 1% Acpr4, 1%Acet4, 10% 4-on, or 10%4-ol on the day of cell grafting and on the two following days. The mice were killed 5 days after tumor cell grafting, 12 lesions from each group (inhaled and non-inhaled) were harvested, and weighed. Index of inhibition("mass index") was calculated dividing mass of tumors in inhaled groups by mean mass of tumors collected from the control group of mice.

Experiments were approved by Local Ethical Committee.

Statistical analysis

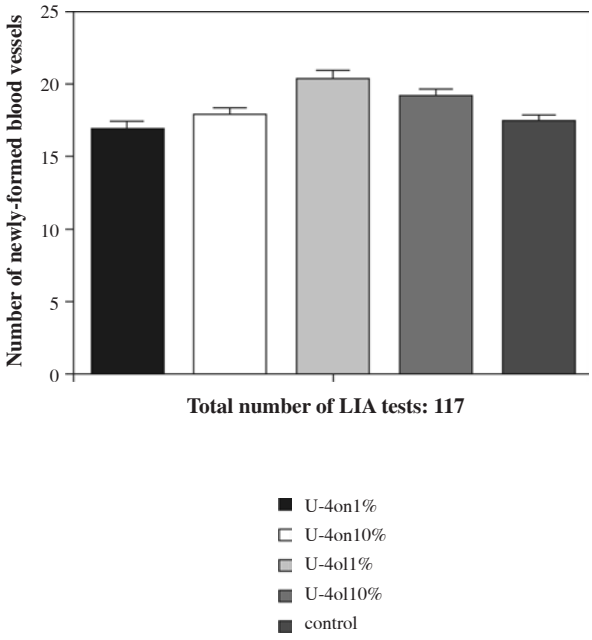
The results of immunological experiments were verified statistically by a one-way ANOVA analysis of variance, some of them by 2-way ANOVA (GraphPad Prism software package)and the significance of differences between the groups was verified with a Tukey's or Bonferroni posttests.

The results of experiments performed with L-1 sarcoma were analyzed by Student *t* test.

Results

Inhalation of mice with 10% undecan-4-ol afteir their contact with antigen ("therapeutic" scheme) resulted in significantly higher neovascular reaction in local graft-versus-host test (LIA) (Fig. 1) and increased antibody production to SRBC (Fig 3) in comparison to the controls. 1% undecan-4-ol was effective in GVH experiment only. Mice inhaled 4-undecanone behaved as the controls in both types of experiments. On the other hand, when BDF1 recipients obtained cells from undecan-4-ol inhaled parental Balb/c mice ("prophylactic" scheme), neovascular reaction was less intense than in the corresponding controls (Fig. 2).

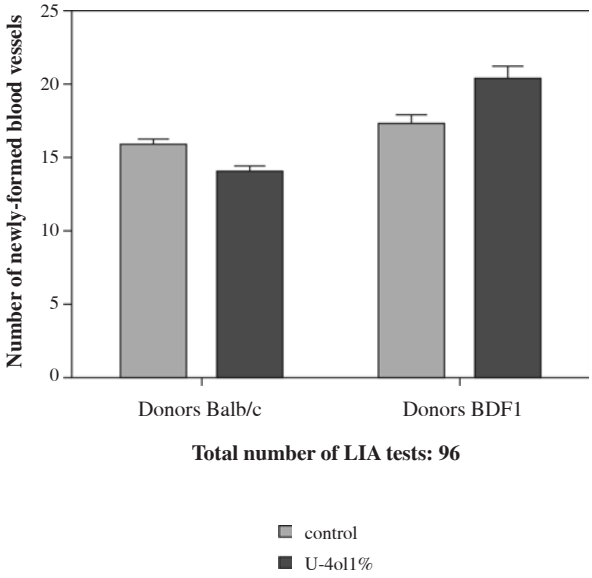
However, when BDF1 hybrids were grafted with lymphocytes obtained from inhaled BDF1 donors, stimulatory



One-way analysis of variance	
p value	<0.0001
p value summary	***
Are means signif. different? (p<0.05)	Yes

Tukey's Multiple Comparison Test	**Mean diff.**	**q**	**Significant? p<0.05?**	**Summary**
U-4on1% vs. U-4on10%	-0.9000	1.822	No	ns
U-4on1% vs. U-4ol1%	-3.500	7.194	Yes	***
U-4on1% vs. U-4ol10%	-2.300	4.656	Yes	*
U-4on1% vs. Control	-0.6000	1.511	No	ns
U-4on10% vs. U-4ol1%	-2.600	5.263	Yes	**
U-4on10% vs. U-4ol10%	-1.400	2.792	No	ns
U-4on10% vs. Control	0.3000	0.7382	No	ns
U-4ol1% vs. U-4ol10%	1.200	2.429	No	ns
U-4ol1% vs. Control	2.900	7.301	Yes	***
U-4ol10% vs. Control	1.700	4.183	Yes	*

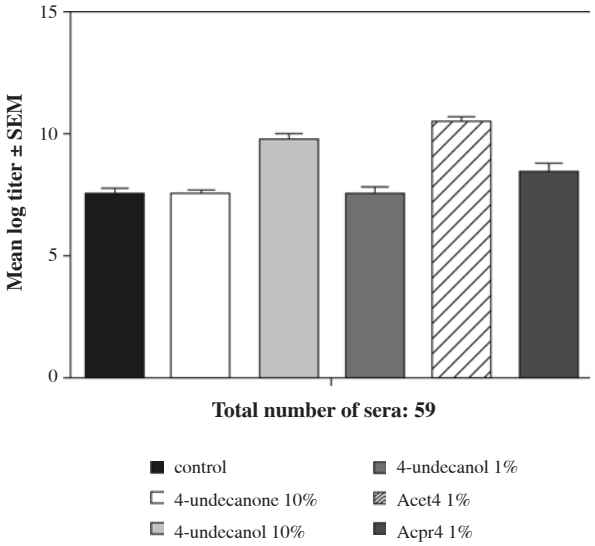
Fig. 1. Neovascular response in BDF1 hybrid mice grafted with parental Balb/c spleen cells and inhaled 4-undecanone or 4-undecanol (mean ± SEM)



Two-way ANOVA		
Source of Variation	**% of total variation**	**p value**
Interaction	12.03	<0.0001
Drug	0.64	0,3197
Cells donor	28.46	<0.0001
Source of Variation	**p value summary**	**Significant?**
Interaction	***	Yes
Drug	ns	No
Cells donor	***	Yes

Bonferroni posttests				
Control vs. 4-ol 1%				
Cells donor	**Difference**	**t**	**p value**	**Summary**
Donors Balb/c	-2.000	3.649	p<0.001	***
Donors BDF1	3.200	2.999	p<0.01	**

Fig. 2. The in vivo influence of 4-undenanol on the angiogenic activity of parental (Balb/c) or syngenetic (BDF1) spleen cells (mean ± SEM)



One-way analysis of variance	
p value	<0.0001
p value summary	***
Are means signif. different? (p<0.05)	Yes

Tukey's Multiple Comparison Test			**Significant? p<0.05?**	
Control vs. 4-undecanone 10%	0.0000	0.0000	No	ns
Control vs. 4-undecanol 10%	-2.200	8.745	Yes	***
Control vs. 4-undecanol 1%	0.0000	0.0000	No	ns
Control vs. Acet4 1%	-2.900	13.96	Yes	***
Control vs. Acpr4 1%	-0.9000	4.333	Yes	*

Fig. 3. The effect of 4-undecanone and its derivatives on anti-SRBC antibody production in mice

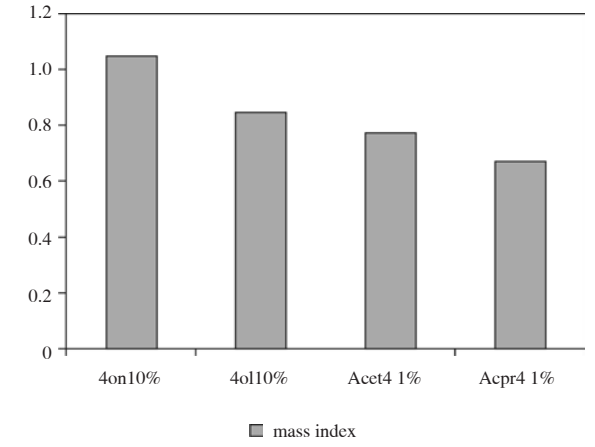


Fig. 4. The effect of inhalations on tumor mass

effect was observed (Fig. 2). No effects of inhaled 4-on were observed.

Inhalation of mice with Acet4 or Acpr4 after SRBC injection significantly increased anti-SRBC antibody level in their sera (Fig. 3).

In experiments with L-1 sarcoma, inhalation of mice with almost all tested compounds, (except 4-undecanone, 4-on),resulted in diminishing of tumor mass (Fig. 4). Mass indices ± SE were: 1.05±0.05 for 4 on (difference non-significant); 0.85±0.05 for 4-ol (difference on the border of statistical significance); 0.78±0.04 for Acet4 ($p<0.05$); 0.67±0.05 for Acpr4 ($p<0.01$).

Discussion

Animals have a well-developed olfactory sensitivity for aliphatic ketones existing in the nature, and, surprisingly, also for the products of chemical synthesis, such as undecan-4-ol and 4-one acetals , what we present in this paper.

Aromatherapy has a long history. Aromatic substances of plant origin were used in ancient China, India, Egypt, for various purposes. Also Greeks and roman physicians recommended fragrant oils for treatment of various diseases.

Some essential oils – among them lavender oil and tea-tree (*Melaleuca alternifolia*) oil, were recognized as strong antimicrobial factors [27].

In our previous studies we presented, for the first time, their strong immunostimulatory properties [28-31]. However, we also discovered, that some other aromatic oils (synthetic almond oil, manuka essential oil) suppressed antibody production in inhaled mice [32].

It was the reason for beginning our study on the positive or negative influence of some flavours (used in food and cosmetic industries) on cellular and humoral immune response.

Recently, we have shown for the first time the in vivo immunostimulatory properties of methyl n-nonyl ketone (2-undecanone), substance present in various plants and foods, on cellular and humoral immunity in mice. The activation of respiratory burst, measured by colorimetric assay(RBA) and activation of phagocytic activity of granulocytes (PKA test), as well as increased blood and splenic lymphocytes response to LPS and ConA were observed in mice inhaled for 3 days with 2on, 5 drops for 5 mice during 1 hour daily. In mice inhaled for 3 days after immunisation with sheep red cells (SRBC) significant stimulation of antibody response was

observed on the day 7th. F1 hybrid mice inhaled for 3 days (days 0, +1 and +2) after intradermal grafting of parental cells presented on the 3rd day significantly more newly-formed blood vessels (immunological angiogenesis) than respective controls. Serum lysozyme level increased in inhaled mice, serum levels of C-reactive protein (CRP), gamma-globulins and ceruloplasmin did not differ from the controls [19]. In the next paper, the in vivo effect of some derivatives of 2-undecanone on humoral immunity in mice were studied. Stimulatory effect on anti-SRBC antibody production was presented by undecan-2-ol, undecan-2-ol R(-) enantiomer, undecan-2-ol acetate and undecan-2-one acetals. No effect of S(+) enantiomer of undecan-2-ol was observed. Serum lysozyme level increased in mice inhaled with undecan-2-ol [20].

We also studied the in vivo effects of 3-undecanone and 3-undecanol on the cellular and humoral immunity in mice. An increase of antibody production and increase of the lysozyme level in sera of mice which inhaled 10% 3-ol were observed. 3-ol inhaled mice presented a higher activation of respiratory burst (RBA test) and phagocytic activity of blood granulocytes (PKA test) than the corresponding controls. In the 3-ol inhaled group the proliferative response of lymphocytes isolated from blood and the spleen to mitogens ConA and LPS was significantly higher than in the controls. Inhalations of mice with a lower concentration (1%) of 3-ol, or with 10% 3-one were ineffective [21]. Intracutaneusly grafted Sarcoma L-1 tumors were significantly smaller in U3-ol inhaled mice than in mice inhaled with U3-one as well as those from the control group. No effect of inhalations with 2-undecanone or its derivatives on L-1 sarcoma growth were seen [32].

In the present paper 4-undecanone, similarly as 3-undecanone, presented no immunotropic or anti-tumor properties, and 4-ol, similarly as 3-ol, stimulated cellular and humoral immunity, and diminished mass of tumors. Also both 4-undecanone acetals stimulated antibody production and influenced tumor growth.

Contrary to the stimulatory effect of 4-ol on G-V-H reaction when inhalations were applied to the recipients of parental lymphocytes, treatment of parental donors resulted in diminished neovascular response. This might be connected with increasing ability to production of angio-inhibitory factors, but it should be elucidated by further experiments.

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