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Melleolides induce rapid cell death in human primary monocytes and cancer cells

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ABSTRACT

The melleolides are structurally unique and bioactive natural products of the basidiomycete genus *Armillaria*. Here, we report on cytotoxic effects of melleolides from *Armillaria mellea* towards non-transformed human primary monocytes and human cancer cell lines, respectively. In contrast to staurosporine or pretubulysin that are less cytotoxic for monocytes, the cytotoxic potency of the active melleolides in primary monocytes is comparable to that in cancer cells. The onset of the cytotoxic effects of melleolides was rapid (within <1 h), as compared to the apoptosis inducer staurosporine, the protein biosynthesis inhibitor cycloheximide, and the DNA transcription inhibitor actinomycin D (>5 h, each). Side-by-side comparison with the detergent triton X-100 and staurosporine in microscopic and flow cytometric analysis studies as well as analysis of the viability of mitochondria exclude cell lysis and apoptosis as relevant or primary mechanisms. Our results rather point to necrotic features of cell death mediated by an as yet elusive but rapid mechanism.

1. Introduction

The melleolides, made by the basidiomycete genus *Armillaria* (honey mushrooms), are a structurally unique family of natural products as they combine orsellinic acid, or a derivative thereof, with a protoilludene-type secondary alcohol.[1,2] The melleolide backbone represents a metabolic hybrid, as both a sesquiterpene cyclase[3] and a polyketide synthase mediate biosynthesis, with the latter cross-linking the building blocks through esterification.[4] Some, but not all melleolides are regioselectively chlorinated at C-6′, as exemplified by arnamial **1** and its 6′-dechloro derivative **2**.

Initially, the melleolides were recognized as antimicrobially active compounds.[5,6] Subsequently, cytotoxic activity against human cancer cell lines was described for **1**[7] which showed an IC_{50} of 3.9 μM for Jurkat cells. First insight into the structure–activity relationship came from a subsequent study which included Jurkat, K-562, HeLa, and MCF-7 cancer cells.[8] The more hydrophilic compounds did not affect cell viability implying that primarily terpene hydroxylation at positions 10, 13, and 14 is critical for cytotoxicity. Consequently, dehydroarmillylorsellinate (**3**) which does not carry an alcohol functionality at any of these positions, was found to be active against K-562 cells (IC_{50} = 5.0 μM).

For antifungal activity, the double bond position ($\Delta^{2,4}$) of the sesquiterpene moiety of melleolides was shown to be critical for antifungal activity. An absent or shifted double bond ($\Delta^{2,3}$), like in armillarin (**4**), armillaridin (**5**), and melleolide D (**6**), leads to a loss of antifungal activity but does not impact cytotoxicity against human cells.[9]

In our present study we compared the cytotoxic activity of melleolides towards proliferating cancer cells and non-transformed primary monocytes. We hypothesized that monocytes would be less susceptible, due to their non-proliferating character and the fact that they are generally less sensitive to most cytotoxic/anti-proliferative agents, as compared to cancer cells. In fact, primary monocytes or peripheral blood mononuclear cells (i.e., monocytes and lymphocytes) are often utilized as models in order to demonstrate cancer cell selectivity of proposed anti-cancer agents. For example, we previously showed that induction of cell death of primary human monocytes by the microtubule-disrupting agent pretubulysin and the apoptosis inducer myrtucommulone required 20- to >100-fold higher concentrations, respectively, as compared to various cancer cell lines.[10,11] Conversely, agents that target specific cell receptors of leukocytes such as leukotoxin from

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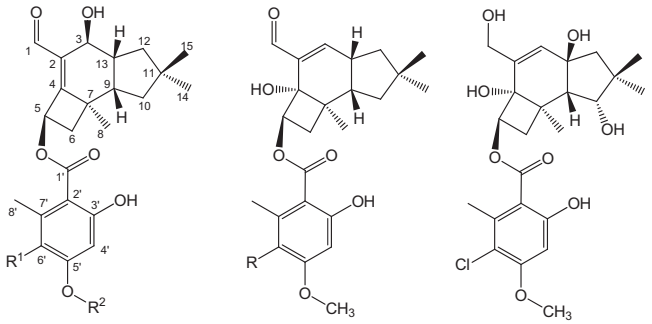

Figure 1. Structures of the melleolides **1**–**6** from *A. mellea*.

Aggregatibacter actinomycetemcomitans may cause selective cytotoxicity against leukocytes without affecting cancer cells.[12–14]

In the present study we screened a set of six melleolides (Fig. 1) with (**1**–**3**) or without (**4**–**6**) antifungal activity for their cytotoxic effects on primary monocytes from human whole blood. Consistent with our previous results for cancer cell lines,[8] we found that the α,β-unsaturated aldehyde and absence of hydroxy groups at the cyclopentene primarily correlated with potent cytotoxic properties towards various cancer cells, but also against primary monocytes.

Intriguingly, we observed an unusually rapid onset of cell death (<1–2 h) for the cytotoxic melleolides, which has not been reported for other well-recognized cytotoxic agents such as staurosporine, cycloheximide, or actinomycin D. Our data suggest a unique mode of action of melleolides regarding their cytotoxicity that warrants further attempts to identify the respective molecular target(s) in future studies.

2. Results and discussion

2.1. Structure–activity relationship of melleolides for induction of cell death

A set of six previously described melleolides (**1**–**6**, Fig. 1)[8,9] was analyzed for cytotoxicity properties against freshly isolated human primary monocytes and a set of cancer cells (i.e., THP-1, Mono Mac 6 (MM6), K-562 and HeLa cells). Employing the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, we determined the lowest EC_{50} of 2.3 μM for **3** regarding the loss of monocyte viability, which is comparable or even lower than the EC_{50} values (1.6–5.0 μM) analyzed for transformed cancer cell lines in this respect (Table 1). Similar results were obtained for **1** and **2**, which share the important structural feature for anti-fungal activity, that is, the $\Delta^{2,4}$-double bond. Melleolides **4** and **5**, which are characterized by a $\Delta^{2,3}$ double bond, were equally active against THP-1 and MM6 cells (EC_{50} values between 4.5 and 6.6 μM), but exerted lower cytotoxic effects against monocytes, HeLa, and K-562 cells (8.9–43.2 μM). Melleolide D (**6**), which features a hydroxy group at C-1, instead of the aldehyde, is essentially inactive (Table 1, Fig. 2A).

The high cytotoxic potential of selected melleolides observed in primary monocytes was also evident in the human leukemia cell lines THP-1 and MM6, implying the presence of a common cellular target in primary monocytes and cancer cells as being responsible for the cytotoxic effects.

Next, we verified the induction of monocytic cell death by measuring LDH levels as marker for loss of membrane integrity in melleolide-treated supernatants. As shown in Figure 2B, marked cytotoxic effects of **3** with the $\Delta^{2,4}$-double bond, but not for the antifungally inactive **6** with its tetrahydroxylated sesquiterpene moiety were evident; staurosporine and triton X-100 were used as reference controls that caused LDH release, as expected.

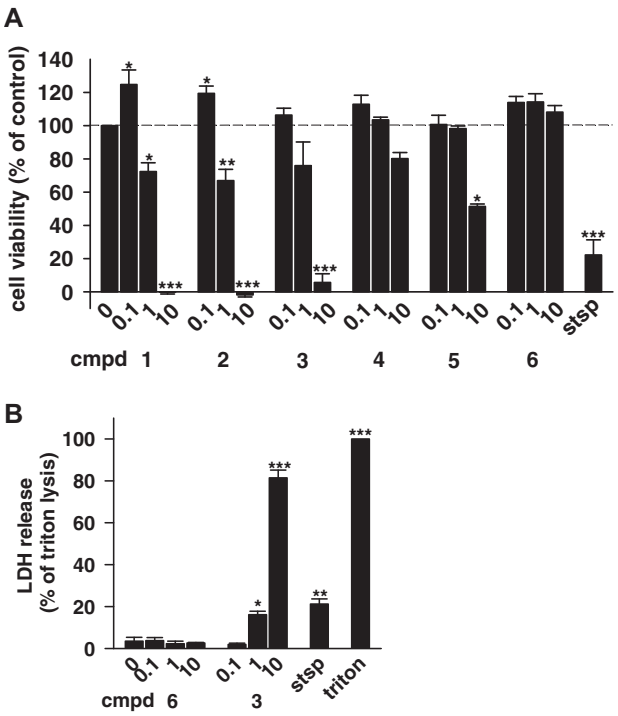

Figure 2. Reduction of monocyte viability by melleolides. (A) Monocytes were incubated with melleolides **1**–**6** at the indicated concentrations (μM) or staurosporine (stsp, 3 μM). Cell viability was analyzed by the MTT assay after 24 h. (B) Monocytes were treated with melleolides **3** and **6** at the indicated concentrations (μM), staurosporine (stsp, 3 μM), and triton X-100 (0.2%), respectively. After 4 h the release of LDH was analyzed. Data are given as mean + SE, $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle control; ANOVA + Bonferroni.

Table 1
Cytotoxic effects of melleolides

Compd	THP-1 EC_{50} (μM)	MM6 EC_{50} (μM)	monoc. EC_{50} (μM)	K-562 EC_{50} (μM)	HeLa EC_{50} (μM)	
1	2.9 (±0.4)	3.2 (±0.4)	3.8 (±0.5)	2.3 (±0.3)	4.9 (±0.2)	$\Delta^{2,4}$-Double bond
2	2.4 (±0.7)	2.9 (±0.7)	3.1 (±0.7)	4.1 (±0.1)	12.3 (±0.3)	
3	3.0 (±0.5)	4.2 (±1.9)	2.3 (±0.4)	5.0 (±0.3)	1.6 (±0.1)	
4	4.8 (±0.5)	6.6 (±0.7)	43.2 (±2.4)	23.7 (±1.5)	16.7 (±2.1)	$\Delta^{2,3}$-Double bond
5	4.5 (±0.1)	5.6 (±0.3)	12.3 (±2.2)	8.9 (±1.3)	9.2 (±1.7)	
6	>100	48.6 (±1.0)	60.7 (±0.7)	>100	>100	

EC_{50} values for induction of cell death of THP-1, Mono Mac 6 (MM6), monocytes (monoc.), K-562, and HeLa cells within 24 h. Data are given as mean ± SE, $n = 3$.