Effects of tetracycline on human monocyte phagocytosis and lymphocyte proliferation

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The phagocytosis and the spreading ability of cultured human monocytes were tested after administration of tetracycline (Tc) in vivo (14 days) and in vitro (24 h, 48 h, 72 h). As compared with untreated monocytes, in vivo medication had negligible effects on the cells' spreading ability and phagocytosis mediated via immune (Fc) and non-specific receptors. Addition of increasing concentrations of Tc in vitro (0–10 μg/ml), chosen to mimic in vivo therapeutic serum values, caused no statistically significant changes in the monocyte functions tested when all data were analyzed together. Regardless of Tc doses used, cultured monocytes showed maximum spreading and phagocytic activities after 48 h in vitro. Corresponding Tc exposure for 48 h in vitro had no effect on phytohemagglutinin-induced lymphocyte thymidine incorporation. □ *Antibiotics; cell culture; lymphocytes; monocytes*

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Tetracycline (Tc) is often prescribed for long-term treatment of infections, including recalcitrant periodontitis. Its effect on the host's immune response remains unclear. Some reports have indicated that Tc suppressed leukocyte chemotaxis when studied in Boyden chambers (1–3), whereas normal chemotaxis was found when using agarose techniques (4). Gangulu et al. (5) found that Tc medication interfered with production of monocyte migration inhibition factors by human lymphocytes and also modulated normal cell migration. Other authors have shown that subliminal amounts of Tc significantly increased phagocytosis of pathogens tested (6, 7). Macrophages showed ability to concentrate Tc intracellularly (6). It has been suggested that the Ca^{2+}-binding effect of Tc might explain the antibiotic's effects on phagocytosis (8, 9). The importance of Tc type and concentration in this respect has been emphasized (8).

Preus & Mörland (10) studied cultured monocytes from two patients with Papillon–Lefèvre syndrome (PLS). We found decreased Fc-receptor-mediated attachment and phagocytosis and reduced lymphocyte proliferative response to phytohemagglutinin (PHA). Since the PLS patients had been taking Tc medication for 18 months, the cellular dysfunctions observed might be a side effect of the medication.

The aim of the present study was to study the effect of Tc on the in vitro spreading ability and receptor functions of monocytes and the lymphocyte blastogenesis in blood mononuclear cells from two healthy blood donors.

Materials and methods

Two 31-year-old healthy men volunteered for the experiments.

Preparation of monocyte and lymphocyte cultures

Mononuclear cells were isolated from ethylenediaminetetraacetic acid (EDTA)-treated venous blood (11). The cells were suspended in RPMI 1640 (Gibco Lab., Grand Island Biological Co., Glasgow, Scotland) supplemented with 10% autologous serum (AS) at 3×10^6 cells/ml and were seeded on coverslips in multiwell Linbro

plates (Linbro Chemical Co., New Haven, Conn., USA). Culturing was performed at 37°C in a mixture of 5% CO_2 in air. After 2 h of incubation the lymphocyte-containing supernatants were harvested and distributed (1.0×10^6 cells/ml) in 96-well microtiter plates (3596, Costar, Cambridge, Mass., USA) with graded doses of Tc. The remaining adherent cells (>95% monocytes) were washed three times in RPMI 1640 and then cultured in 1 ml of fresh serum-containing medium, supplemented with increasing doses of Tc.

Tetracycline administration in vitro

Tetracycline® (TcHCl) powder (Apothekernes Laboratorium A/S, Oslo, Norway) was disolved in sterile, physiological saline. Appropriate portions of Tc were added to the monocyte cultures, yielding final concentrations of 1, 3, 5, and 10 μg/ml. Then the cells were cultured for 24 h, 48 h, and 72 h, respectively. The lymphocytes were exposed to the same Tc concentrations and cultured for 48 h.

Tetracycline administration in vitro

One male test person (weight, 85 kg) received a daily dose of 1 g (500 mg × 2) Tc for 14 days between two blood samplings. The autologous cells from this person, obtained before Tc medication, served as reference. Monocytes were isolated as described above and assayed after 24 h in culture without addition of Tc.

Microscopy

The monocyte monolayers were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) with 0.1 M sucrose. The coverslips were mounted on a drop of Aquamont (Edward Gurr Ltd., London). Cell morphology and phagocytosis were evaluated in a Zeiss phase contrast microscope. Ten fields were randomly selected from each coverslip specimen, and the cells in them counted. About 500 cells per coverslip were thus counted.

The proportion of cells showing cytoplasmic veils and/or extensions (spreading ability) was used as indicator of monocyte activity (12).

The function of the Fc receptors was assayed by the monocytes' ability to attach and internalize IgG-sensitized sheep erythrocytes (EA) (13). When two or more EA were directly associated with the monocyte surface, a positive attachment score was recorded. Internalization was deemed positive when more than one EA was visible inside one monocyte.

Non-specific phagocytosis was assessed by adding latex particles (0.04%) to cultures, as described by Mørland & Kaplan (13).

Lymphocyte blastogenesis

Lymphocytes cultured in microtiter plates were pulsed with ^{3}H-thymidine (2 μCi/well in aliquots of 5 μl) 16 h before harvesting, as described by Tollefsen et al. (14). Each well contained 10^5 cells in 0.1 ml medium, 0.5, 0.1, and 0.5 μg/ml PHA in aliquots of 0.05 ml medium (PHA, HA 16, Wellcome Research Laboratories, Beckenham, U.K.), and graded concentrations of Tc (1, 3, 5, and 10 μg/ml). Culture medium was RPMI 1640 containing 25 mM Hepes buffer and 10% AS.

Methodologic considerations

Three coverslips showing monocyte spreading ability and attachment and internalization of EA, respectively, were randomly selected. Each coverslip was assessed in the microscope for those variables in 20 randomly chosen fields. Then the microscopic fields were photographed and the same variables assessed on the photographs by the same examiner after 7 and 14 days.

Assuming that the countings in the microscope represented 'true values' (that is, 100% of all cells with the characteristic in question), the reproducibility, as judged by the help of photographs, can be expressed as percentage of 'true values' obtained. The reproducibility of the method, expressed as percentage identical figures from the countings of the same photographs, exceeded 90%.

Table 1. Attached and internalized IgG sensitized sheep erythrocytes (EA) mediated by Fc receptors in monocytes* exposed to tetracycline in vitro

Tetracycline conc. (daily addition)	Percentage monocytes associated with EA											
	24 h				48 h				72 h			
	Attached		Internalized		Attached		Internalized		Attached		Internalized	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0 µg/ml	51.8	3.5	32.8	6.9	87.1	6.1	49.5	10.5	44.3	11.4	25.7	11.1
1 µg/ml	61.8	4.6	33.1	1.9	92.3	0.8	45.1	9.2	64.5	2.5	22.7	8.6
3 µg/ml	70.0	4.4	29.4	0.7	91.0	1.3	28.5	3.7	63.0	10.0	14.6	2.6
5 µg/ml	62.4	2.1	34.8	8.7	91.1	2.7	30.9	3.5	62.0	7.0	13.6	2.9
10 µg/ml	57.0	5.0	26.8	0.2	91.4	1.8	20.4	2.1	47.1	3.8	10.4	2.9

* The cells originated from two test persons and were obtained before the in vivo Tc medication experiments.

Statistical evaluation

Regression analyses were used on the results given in Table 1. Analysis of variance was performed on the data in Table 3, a G test of independence was used for Table 4; all tests were performed as recommended by Sokal & Rohlf (15). The chosen level of significance was 0.01.

Results

The Fc-receptor-mediated attachment of monocytes was slightly increased when exposed to Tc in vitro (Table 1). The increased values were valid for monocytes cultured for 24 or 72 h with Tc added but not for cells similarly cultured for 48 h (Table 1). When regression analysis was applied on the empirical data, only the time factor reached statistical significance. Attachment was significantly increased in all specimens after 48 h in culture (attachment = 0.874 days^2; $F = 48.30$; $p < 0.01$, standardized form).

Fc-receptor-mediated internalization of monocytes was not influenced by Tc exposure for 24 h in vitro (Table 1). After 48 to 72 h in vitro internalization decreased dose-dependently (Table 1). When all data were analyzed together, the variation ascribed to Tc exposure was not statistically significant. Internalization was, however, significantly influenced by culture time and test persons (internalization = −0.62 day − 0.41 person; $F = 17.62$; $p < 0.01$, standardized form).

Monocyte spreading ability was not significantly ($p < 0.01$) altered by addition of 1–10 µg/ml Tc in vitro (Table 2). Nor did Tc medication in vivo influence the monocyte spreading ability (data no shown). Nonspecific phagocytosis of latex particles was not altered in the presence of Tc, all observations being close to 100% (data not shown).

DNA incorporation of ^{3}H-thymidine in lymphocytes was not significantly altered in the presence of Tc in concentrations ranging from 1 to 10 µg/ml (Table 3).

Tc medication in vivo produced insig-

Table 2. Spreading ability of human monocytes* exposed to tetracycline in vitro

Tetracycline (daily addition)	Percentage cells					
	24 h		48 h		72 h	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
0 µg/ml	98.3	1.0	95.8	1.5	96.6	0.4
1 µg/ml	96.2	0.5	96.1	1.6	93.7	3.3
3 µg/ml	95.6	3.1	95.8	3.2	92.2	4.0
5 µg/ml	96.2	2.7	93.6	1.4	95.0	0.5
10 µg/ml	96.7	2.1	86.1	4.4	81.5	7.5

* See Table I.

Table 3. Incorporation of radioactive thymidine in PHA-stimulated human lymphocytes* exposed to tetracycline in vitro

Tetracycline (initial addition)	Incorporation of ^{3}H-thymidine (in cpm)†			
	0 µg PHA/ml	0.05 µg PHA/ml	0.10 µg PHA/ml	0.50 µg PHA/ml
0 µg/ml	90.1	93.5	220.1	2034.3
1 µg/ml	86.8	121.9	260.0	2038.0
3 µg/ml	76.8	134.2	264.3	2103.5
5 µg/ml	76.9	119.6	236.6	1876.1
10 µg/ml	70.8	103.2	237.8	2086.8
25 µg/ml	72.6	99.3	249.4	1579.5

* See footnote to Table 1.

† The data (y) were transformed $y' = (E\text{-}C)^{\frac{1}{2}}$, where E represents the lectin-stimulated cultures, and C the unstimulated cultures, before analysis of variance. The dose-response effect of PHA was statistically significant ($F = 851.53$; $p < 0.01$), whereas Tc produced no significant influence on the ^{3}H-thymidine uptake ($F = 1.54$; NS).

nificant changes in monocyte ability to attach and internalize sensitized particles (Table 4). No statistically significant differences were found between the two blood donors, tested in parallel (Table 4). Internalization of test particles was slightly increased in monocytes isolated from the test person given Tc medication (Table 4), but the difference did not reach a statistically significant level.

Discussion

The generally accepted therapeutic concentration of tetracycline in serum is 0.5–1.5 µg/ml (9, 16, 17). However, serum values up to 4 µg/ml have been reported (18). The present in vitro drug concentrations were chosen in accordance with these values. We found that Tc added to cultured mononuclear cells did not significantly affect monocyte function (Tables 1 and 2). Nor did relevant concentrations of Tc affect lymphocyte blastogenesis. The modest isotope uptake by lymphocytes could reflect a separation procedure that was close to 100% effective.

Intermediate concentrations of Tc in vitro (3–5 µg/ml) caused increased attachment of opsonized test particles (Table 1). These results agree with those studies showing enhanced phagocytic activity in mouse macro-

Table 4. Attached and internalized IgA-sensitized sheep erythrocytes mediated by Fc receptors in human monocytes exposed to tetracycline in vivo

Cell	Tetracycline administration	Percentage of cells							
		Day 0				Day 14			
		Attached*		Internalized†		Attached*		Internalized†	
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
1	0 g/day	55.3	4.0	39.1	6.1	83.5	2.9	71.0	4.0
2	0 g/day	48.4	1.4	26.1	1.0	75.4	1.2	73.7	2.8
2	1 g/day								

* $G = 0.015$; † $G = 2.154$ (NS).

phages exposed to Tc in suboptimal doses (6, 7). However, when the data were analyzed together (Table 1), statistical signficance was not reached. Cultures containing intermediate and high concentrations of Tc for more than 24 h in vitro expressed decreased Fc-receptor-mediated internalization (Table 1). Ca^{2+} ions are necessary for internalization of particles by this receptor (19, 20), suggesting that high concentrations of Tc caused binding of Ca^{2+} in the culture media, followed by suppressed internalization. On the other hand, reports have shown that tetracycline levels in gingival exudate may be four times higher than in serum after systemic Tc administration (21, 22). These Tc concentrations may therefore reach levels sufficient to suppress leukocyte function in the local tissues. However, such high concentrations would hardly be attained in peripheral blood in vivo.

Statistically significant differences among culture times, with regard to both attachment and internalization, were consistently observed (Table 1). The deviation might be caused by suboptimal opsonization of the test particles used after 24 and 72 h in vitro (see controls in Table 1) and on day 0 in vivo (Table 4). Such test particles are probably well suited to detect small differences between Tc-modified cells and control cells (Table 1). However, to judge from previous studies (23) and our own unpublished data, we believe that maximum numbers of Fc receptors are expressed after 2 days in culture.

Administration of high Tc doses (1 g/day to an adult person of 85 kg) in vivo for 2 weeks produced no inhibitory drug effects on the monocyte functions tested. Yet, the very low number of test persons might have masked subtle differences. However, we felt it unethical to recruit healthy persons and give them a 1-g/day Tc medication for 14 days. Neither could we use persons who for one reason or another were taking a Tc medication, because their general disease might include altered mononuclear blood cell functions. Moreover, it cannot be ruled out that some of the variation in monocyte functions was caused by factors other than Tc mediation during the test period (24–26). A contribution of physical and psychological stress is possible, although less likely.

Other reports have shown that chlortetracycline inhibits phagocytosis and production of migration inhibitory factor. Recent work showed that tetracycline hydrochloride also triggers such effects (9; A. Ness, personal communication). The antibacterial activities of the various tetracyclines are, however, comparable (16). We chose to test tetracycline hydrochloride (Tetracyclin®, Apothekernes Laboratorium for Specialpræparater, Oslo, Norway) because it had been administered to two PLS patients with deficient monocyte Fc-receptor function (10). The present results suggest that the monocyte dysfunctions found in those PLS patients were not caused by the Tc regimen used. Admittedly, long-term use (18 months) of a drug might produce results not detectable after 2 weeks. Whether the monocyte defects in those PLS patients reflect reduced production of cytokines or genetic abnormalities or were induced by the local microbial flora remains to be examined. Since Tc can accumulate intracellularly up to 60–80 μg/ml in human leukocytes (6), a study of local cells might prove more affirmative in studies of periodontitis.

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