Effects of Waterborne Chloramphenicol and Oxytetracycline Exposure on Haematological Parameters and Phagocytic Activity in the Blood of Koi Carp, *Cyprinus carpio*

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ABSTRACT

Chloramphenicol and oxytetracycline are used in ornamental fish culture to treat bacterial infections. The present study was aimed at evaluating the effects of different concentrations of chloramphenicol (2-10 mg L⁻¹ for 10 days) and oxytetracycline (20 mg L⁻¹ for 3 and 10 days, 100 mg L⁻¹ for 3 days) on hematological parameters and phagocytic activity in the blood of koi carp, *Cyprinus carpio*. Results showed that treatment of fish with 10 mg L⁻¹ chloramphenicol for 10 days depressed haematocrit, erythrocyte counts and mean corpuscular volume leading to anaemia. It also induced leucocytosis coupled with neutrophilia, thrombocytosis and lymphocytosis. The phagocytic index of the fish was also enhanced significantly with respect to the controls. The treatment of fish with 100 mg L⁻¹ oxytetracycline for 3 days induced leucopenia coupled with neutropenia. Phagocytic index of these fish was significantly depressed compared to the controls. Results revealed that precautions should be taken when high concentrations of chloramphenicol or oxytetracycline are used in koi carp culture especially for long term treatments.


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INTRODUCTION

Chloramphenicol and oxytetracycline are two antibiotics used in aquaculture to treat bacterial infections in fish. These antibiotics are suitable as injections, orally medicated food and as immersions (Schaperclaus et al., 1991). Immersion concentrations of chloramphenicol commonly practiced in finfish culture include 2 mg L\(^{-1}\) (Subasinghe, 1992) and 5 to 10 mg L\(^{-1}\) (Supriyadi and Rukyani, 1992). An immersion concentration of oxytetracycline recommended for finfish culture varies from 10 mg L\(^{-1}\) to 100 mg L\(^{-1}\) (Noga, 2000).

The use of chloramphenicol is restricted in food animals (Noga, 2000). It causes idiosyncratic aplastic anemia in humans. The CONH group of chloramphenicol is suspected for the bone marrow suppression in the process of haemopoiesis in mammals (Brander et al., 1991). Interperitonial injections of chloramphenicol caused suppression of erythroblast numbers in the peripheral blood of European eel (Anguilla anguilla). In these fish, the heterophils and thrombocytes dropped but monocytes, basophils and lymphocytes elevated in the peripheral blood whereas phagocytosis of monocytes were stimulated three days after chloramphenicol injection (Kreutzmann, 1977). Rijkers et al. (1980) reported that oxytetracycline injections (180 mg kg\(^{-1}\)) for three days caused alteration of several haematological parameters in koi carp and suppressed the immune response in fish.

The present study was aimed at evaluating the effects of prolonged exposure by therapeutic immersion to different concentrations of chloramphenicol and oxytetracycline on the primary and secondary erythrocyte indices, total and differential leukocyte counts and phagocytic activity in the circulating blood of koi carp, Cyprinus carpio.

MATERIALS AND METHODS

Test fish

Apparently healthy koi carp of 30-70 g in body weight and 12-15 cm in total length were obtained from a commercial ornamental fish farm. The fish were acclimated to laboratory conditions in glass aquaria filled with aged, aerated tap water under the natural photoperiod for 14 days. During the acclimation period, the fish were fed once daily with commercially prepared fish feed (Prima, Sri Lanka) \textit{ad libitum}.

Chloramphenicol and Oxytetracycline treatment Chloramphenicol manufactured by State Pharmaceuticals Company in Sri Lanka and Oxytetracycline (TETRAN-VET\textsuperscript{®}) from Unjha Formulations Limited, India were used in this study. The treatment concentrations of chloramphenicol (2, 5 and 10 mg L\(^{-1}\)) and oxytetracycline (20 and 100 mg L\(^{-1}\)) were prepared by diluting appropriate amounts of antibiotics in separate glass aquaria containing aged tap water.

For the chloramphenicol exposure, three sets of glass aquaria each filled with 45 L of specific concentrations of chloramphenicol (2, 5 and 10 mg L\(^{-1}\)) in aged tap water were used. Four fish were introduced to each aquarium and the treated waters were continuously aerated using air pumps. Fish maintained in another set of glass aquaria each filled with 45
L of aged, aerated tap water at comparable densities served as controls. Control fish and fish exposed to chloramphenicol were fed once daily with commercially prepared fish feed at 1% of body weight. The treated water and water in the control aquaria were renewed every three days. After continuous exposure to chloramphenicol for 10 days, the exposed fish and control fish were sacrificed (n = 8) for blood sampling.

The same treatment procedure was followed for exposure of fish to 20 mg L\(^{-1}\) and 100 mg L\(^{-1}\) oxytetracycline for 3 days. In addition, a sample of acclimated fish were maintained in another set of glass aquaria each filled with 45 L of 20 mg L\(^{-1}\) oxytetracycline for 10 days. The fish maintained in aged tap water at comparable densities served as controls. Fish were fed daily at 1% of body weight and water was continuously aerated. The aquaria were covered with black coloured paper to minimize the photoinactivation of oxytetracycline. After the specified oxytetracycline exposure, fish exposed to oxytetracycline and control fish (n = 8) were sacrificed for blood sampling.

**Physico-chemical parameters in aquaria water**

During the exposure period, pH, temperature and dissolved oxygen concentration (DO) were measured daily using water quality monitors (HI 8314 membrane pH meter HANNA\(^{\circledR}\) instruments, TOA\(^{\circledR}\), WQC-22A water quality checker).

**Haematological parameters**

Fish were sacrificed by pithing and blood samples were taken by severing the caudal vein of the fish. Before drawing the blood, pipettes were washed with heparin solution (Heparain Leo\(^{\circledR}\), Leo Pharmaceuticals, Denmark) to delay blood coagulation. Haematocrit and leukocrit values in the blood samples were determined after centrifuging the blood in heparinized microcapillary tubes at 5000 rpm for 5 minutes using a haematocrit centrifuge. The microhaematocrit reader was used to measure haematocrit values whereas the heights of the packed leucocytes were measured using a calibrated micro-eyepiece under the light microscope for determination of leukocrit values.

Erythrocyte count and leukocyte count in the blood samples were determined using Shaw’s solutions as a diluting fluid (Hesser, 1960). Haemoglobin concentration in the blood was determined using cyanohaemoglobin method using Sigma\(^{\circledR}\) test kits (Sigma, MO, USA). Using the haematocrit, erythrocyte count and hemoglobin data, the secondary erythrocyte indices namely Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated as described by Houston (1990).

For the determination of abundance of different leukocytes, blood smears of each fish were stained with Wright’s and Giemsa as described by Chinabut et al. (1991). Neutrophils, monocytes, lymphocytes and thrombocytes in the blood smears were identified as described by Hibiya (1982) and Chinabut et al. (1991). Abundance of each cell type was calculated using the total leukocyte count and percentages of each cell types in the blood smears. At least two blood smears were prepared from each fish.
Phagocytic activity

Phagocytic activity of neutrophils and monocytes in blood was determined as described by Anderson and Siwicki (1995) using *Staphylococcus aureus* (Sigma, MO, USA) with slight modifications. A sample (0.1 mL) of blood was placed in a microtiter plate well, 0.1 mL of *S. aureus* $1 \times 10^7$ cells suspended in phosphate buffered saline at pH 7.2 was added and then mixed well. The bacteria–blood solution was incubated for 25 minutes at room temperature. Five µL of this solution was taken on to a clean glass slide and a smear was prepared. The smear was air dried, fixed with ethanol (95%) for 5 min, air dried and stained with Giemsa for 10 min. Duplicate smears were made from each fish. A total of 100 neutrophils and monocytes from each smear were observed under the light microscope and the number of phagocytizing cells and the number of bacteria engulfed by the phagocyte were counted. Phagocytic capacity and phagocytic index were calculated as follows: Phagocytic capacity equals the number of bacteria engulfed cells divided by the total number of neutrophils and monocytes (phagocytes) examined. Phagocytic index is expressed as the total number of bacteria engulfed by the phagocytes, divided by the total number of phagocytes containing engulfed bacteria.

Statistical analysis

The data are presented as mean ± standard deviation of the mean for each group. For each antibiotic exposure, erythrocytic and leucocytic parameters and phagocytic activities in the blood of control fish and antibiotic exposed fish were compared using one-way analysis of variance (ANOVA). Where differences were significant, Tukey’s test was used in multiple comparisons of the means. The accepted level of significance was p < 0.05 (Zar, 1999).

RESULTS

Temperature (27-29 °C), pH (6.5 – 7.0) and DO (5 - 6 mg L$^{-1}$) in the aquaria water were within favorable limits for fish during the study period. No mortality of Koi carp occurred during chloramphenicol or oxytetracycline exposure. However the fish exposed to 100 mg L$^{-1}$ oxytetracycline for 3 days displayed anorexia.

Effect of chloramphenicol or oxytetracycline exposure on erythrocytic indices

Erythrocyte indices in the blood of control fish and fish exposed to chloramphenicol are presented in Figure 1. The haematocrit values of fish exposed to 10 mg L$^{-1}$ chloramphenicol for 10 days (mean value 32.6%) were significantly lower than that of the control (mean value 44.3%) and the fish exposed to 5 mg L$^{-1}$ chloramphenicol (mean value 43.8%). The erythrocyte counts and MCV in the blood of fish exposed to 10 mg L$^{-1}$ chloramphenicol (mean values $1.84 \times 10^6$ cells mm$^{-3}$, and $1.59 \times 10^{-4}$ fl respectively) was significantly lower than that of the controls (mean values $2.21 \times 10^6$ cells mm$^{-3}$, and $2.28 \times 10^{-4}$ fl respectively), and the fish exposed to lower concentrations of chloramphenicol (mean values 2.12-2.27 $\times 10^6$ cells mm$^{-3}$, and 2.10-2.12 $\times 10^{-4}$ fl respectively). Haemoglobin concentration and MCH value in the blood of fish exposed to 2 mg L$^{-1}$ chloramphenicol (mean values 13.8
g dL\(^{-1}\) and 6.81 x 10\(^{-5}\) pg respectively) were lower than that of the control group (mean values 18.48 g dL\(^{-1}\) and 8.94 x 10\(^{-5}\) pg respectively). However, MCHC value in fish exposed to chloramphenicol was not significantly different from that of controls. None of the oxytetracycline treatments used in this study had any significant effect on primary and secondary erythrocytic indices of the fish (results not shown).

**Figure 1.** Effect of exposure of koi carp (*Cyprinus carpio*) to different concentrations of chloramphenicol for 10 days on erythrocytic indices in the blood. Results are presented as mean and standard deviation, n = 8 per group. For each parameter, bars indicated with different letters are significantly different from each other (ANOVA, Tukey’s test, P<0.05)
Effect of chloramphenicol or oxytetracycline exposure on leukocytes in the blood

Leukocrit levels and total leukocyte counts in the blood of control koi carp and fish exposed to chloramphenicol or oxytetracycline are presented in Figure 2. The leukocrit in the blood of fish exposed to 5 mg L⁻¹ (mean value 2.27%) or 10 mg L⁻¹ (mean value 2.50%) chloramphenicol was significantly higher than that of the control group (mean value 1.45%) and fish exposed to 2 mg L⁻¹ (mean value 1.1%) chloramphenicol. Total leukocyte count of fish exposed to 10 mg L⁻¹ chloramphenicol (mean value 1.9 x 10⁴ mm⁻³) was significantly higher than that of the control group (mean value 1.1 x 10⁴ mm⁻³) and the fish exposed to 2 mg L⁻¹ chloramphenicol (mean value 0.95 x 10⁴ mm⁻³). The leukocrit and total leukocyte count of fish exposed to 100 mg L⁻¹ oxytetracycline for 3 days (mean values 0.64% and 0.91 x 10⁴ mm⁻³ respectively) was reduced in comparison to that of the control fish mean values 0.88% and 1.47 x 10⁴ mm⁻³ respectively and the fish exposed to other concentrations of oxytetracycline (mean values 1.01-1.04% and 1.5-1.55 x 10⁴ mm⁻³, respectively).

Absolute counts of neutrophils, monocytes, lymphocytes and thrombocytes in the blood of chloramphenicol-exposed fish and respective controls are presented in Figure 3. The absolute neutrophil counts and thrombocyte counts in the blood of fish exposed to 5 (mean values 3.9 x 10³ mm⁻³ and 5.19 x 10² mm⁻³ respectively) or 10 mg L⁻¹ (mean values 4.3 x 10³ mm⁻³ and 5.52 x 10² mm⁻³ respectively) chloramphenicol were significantly higher than that of fish exposed to 2 mg L⁻¹ chloramphenicol (mean values 2.07 x 10³ mm⁻³ and 1.24 x 10² mm⁻³ respectively) and controls (mean values 3.32 x 10³ mm⁻³ and 2.1 x 10² mm⁻³ respectively). There was no significant difference in absolute monocyte counts in the blood among experimental and control groups. The absolute counts of lymphocytes in fish exposed to 10 mg L⁻¹ chloramphenicol (mean value 12.5 x 10³ mm⁻³) were significantly higher than that of fish exposed to 2 mg L⁻¹ chloramphenicol (mean value 6.67 x 10³ mm⁻³) and controls (mean value 7.55 x 10³ mm⁻³).

Absolute counts of neutrophils, monocytes, lymphocytes and thrombocytes in the blood of oxytetracycline- exposed fish and controls are presented in Figure 4. Absolute neutrophil count in the blood of fish exposed to 100 mg L⁻¹ oxytetracycline (mean value 0.89 x 10³ mm⁻³) for 3 days was significantly lower than that of the control fish (mean value 2.84 x 10³ mm⁻³) and the fish exposed to 20 mg L⁻¹ oxytetracycline for 3 days (mean value 3.13 x 10³ mm⁻³). There was no significant difference in the absolute monocyte, lymphocyte and thrombocyte counts in the blood of oxytetracycline-exposed groups compared to the control group.

Effect of chloramphenicol or oxytetracycline exposure on phagocytic capacity and phagocytic index in the blood

The effect of chloramphenicol exposure on phagocytic capacity and phagocytic index in the blood of koi carp are presented in Table 1. The phagocytic index of fish exposed to 10 mg L⁻¹ chloramphenicol (mean value 3.9) was significantly higher than that of the control fish (mean value 3.1), and fish exposed to 2 and 5 mg L⁻¹ chloramphenicol (mean values 2.7-2.8). However the phagocytic capacity in the blood of fish exposed to chloramphenicol
Table 1. Effect of exposure of koi carp (Cyprinus carpio) to different concentrations of chloramphenicol for 10 days on phagocytic capacity and phagocytic index in the blood.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Phagocytic capacity</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>97.1 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chloramphenicol-exposed</td>
<td></td>
<td></td>
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<tr>
<td>2 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>94.5 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>94.7 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7 ± 0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10 mg L&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>95.2 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are presented as mean and standard deviation, n = 6-8 per group. In each column, means indicated with different superscripts are significantly different from each other (ANOVA, Tukey’s test, P < 0.05).

was not significantly different from the controls. With respect to the phagocytic capacities in the fish exposed to oxytetracycline, there were no significant differences among experimental and control groups (Table 2). However, the phagocytic index of fish exposed to 100 mg L<sup>-1</sup> oxytetracycline for 3 days (mean value 3.5) was significantly lower than that of the other groups of fish (mean values 4.1-4.4).

DISCUSSION

Chloramphenicol and oxytetracycline are antibiotics used to control bacterial infections in aquaculture (Subasinghe, 1992; Supriyadi and Rukyani, 1992; Tonguthai and Chanratchakool, 1992). Even though they are used as chemotherapeutic agents, these antibiotics could adversely affect the health status of the fish. Chloramphenicol is a highly penetrable antibiotic (Brander et al., 1991). In the present study, chloramphenicol exposure (10 mg L<sup>-1</sup>) for 10 days had a significantly negative effect on haematocrit, erythrocyte counts and MCV in the circulating blood of koi carp. This could lead to anaemia. However haemoglobin content, MHC and MCHC levels in the blood were not significantly affected by 10 mg L<sup>-1</sup> chloramphenicol treatment. The reduced MCV in fish exposed to 10 mg L<sup>-1</sup> chloramphenicol indicate the presence of immature erythrocytes in the circulating blood. Rijkers et al., (1980) found that high doses of oxytetracycline injections (180 mg kg<sup>-1</sup>) could inhibit the erythropoiesis of common carp (Cyprinus carpio). The present study found that exposure of koi carp to oxytetracycline had no significant effects on erythrocyte indices in the blood.

Kreutzmann (1977) found the percentage of heterophiles (neutrophil) and thrombocytes percentage were dropped, monocytes and lymphocytes percentages were increased in kidney and peripheral blood of European eel (Anguilla anguilla) after chloramphenicol injections (repeated injections 2 mg / 100 g dose rate at 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> day). In the present study, total leukocyte counts in the blood were significantly increased in koi carp exposed
Figure 2. Effect of exposure of koi carp (Cyprinus carpio) to different concentrations of chloramphenicol for 10 days or oxytetracycline for 3 and 10 days on leukocrit and total leucocyte counts in the blood. Results are presented as mean and standard deviation, n = 8 per group. For each parameter, bars indicated with different letters are significantly different from each other (ANOVA, Tukey’s test, P<0.05)

Kreutzmann (1977) reported that no significant reduction of granulocytes occurred after oxytetracycline injection (repeated injections 2 mg / 100 g dose rate at 1st, 3rd and 5th day) in European eel (Anguilla anguilla). The present study found that exposure of koi carp to high concentration of oxytetracycline (100 mg L⁻¹ for 3 days) could reduce
Figure 3. Effect of exposure of koi carp (Cyprinus carpio) to different concentrations of chloramphenicol for 10 days on absolute counts of neutrophils, monocytes, lymphocytes and thrombocytes in the blood. Results are presented as mean and standard deviation, n = 8 per group. For each parameter, bars indicated with different letters are significantly different from each other (ANOVA, Tukey’s test, P<0.05)

the leukocyte counts and absolute number of neutrophil counts in the blood leading to leucopenia coupled with neutropenia. It indicates that high doses of oxytetracycline could induce suppression of leukocytes in the blood stream of this fish. Neutrophils released to the blood stream of the fish may be reduced by high doses of oxytetracycline. However oxytetracycline exposure doesn’t seem to affect the release of thrombocytes to the blood stream of koi carp as the absolute thrombocyte count was not changed in fish exposed to oxytetracycline immersions. Rijkers et al., (1980) also reported that parental administration of oxytetracycline (180 mg kg⁻¹) did not affect the thrombocyte counts in common carp (Cyprinus carpio).

Kreutzmann (1977) found stimulated phagocytic activity of monocytes in peripheral blood and spleen after chloramphenicol injections (repeated injections 2 mg/100 g dose rate at 1st, 3rd and 5th day) in European eel (Anguilla anguilla). In the present study, the phagocytic
Table 2. Effect of exposure of koi carp (*Cyprinus carpio*) to different concentrations of oxytetracycline on phagocytic capacity and phagocytic index in the blood.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Phagocytic capacity</th>
<th>Phagocytic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>93.1 ± 4.5(^a)</td>
<td>4.1 ± 0.5(^a)</td>
</tr>
<tr>
<td>Oxytetracycline-exposed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 mg L(^{-1}) for 3 days</td>
<td>94.7 ± 4.5(^a)</td>
<td>4.4 ± 0.6(^a)</td>
</tr>
<tr>
<td>20 mg L(^{-1}) for 10 days</td>
<td>97.8 ± 2.7(^a)</td>
<td>4.2 ± 0.4(^a)</td>
</tr>
<tr>
<td>100 mg L(^{-1}) for 3 days</td>
<td>94.4 ± 2.6(^a)</td>
<td>3.5 ± 0.2(^b)</td>
</tr>
</tbody>
</table>

Results are presented as mean and standard deviation, \(n = 6\) per group. In each column, means indicated with different superscripts are significantly different from each other (ANOVA, Tukey’s test, \(P < 0.05\)).
index in the blood of koi carp exposed to 10 mg L\(^{-1}\) chloramphenicol was significantly increased in comparison to the controls. The elevated phagocytic index may be linked with the elevated neutrophil count in the blood. The leukocytes, especially absolute neutrophil counts were high in fish exposed to 10 mg L\(^{-1}\) chloramphenicol. Evelyn (2002) reported that neutrophils could enhance the phagocytic activity of monocytes. It may also be a result of the acquisition of myeloperoxidase activity from the neutrophils. The increase in cellular elements of the immune system in fish may have contributed to the stimulated phagocytosis observed in these fish.

Tafalla et al. (1999) described the non-specific functions of the head kidney macrophages (phagocytosis and respiratory burst) were not suppressed in fish when oxytetracycline was administered in vivo (medicated feed, 200 mg oxytetracycline kg\(^{-1}\) day\(^{-1}\) and 20 mg L\(^{-1}\) for 2 hours bath treatment) to turbot (Scophthalmus maximus). In the present study, the phagocytic index was reduced but phagocytic capacity was not affected in koi carps exposed to 100 mg L\(^{-1}\) oxytetracycline for 3 days. Phagocytic index indicates the total number of bacteria engulfed by the phagocytes, in relation to the total number of phagocytes containing engulfed bacteria. Phagocytic capacity is expressed as the number of bacteria engulfed cells in relation to the total number of examined phagocytes which include both neutrophils and monocytes. Reduction in phagocytic index in fish exposed to the highest concentration of oxytetracyclcin may have resulted due to oxytetracycline induced suppression of total neutrophils in the blood stream. Neutrophils could enhance the phagocytic activity of monocytes (Evelyn, 2002). Reduction of neutrophils in the blood of koi carp exposed to 100 mg L\(^{-1}\) oxytetracycline may have suppressed their positive effect on the phagocytic activity of monocytes which resulted in the reduced phagocytic index of these fish compared to the controls.

**CONCLUSIONS**

The present study evaluated the effects of different concentrations of chloramphenicol and oxytetracycline given for different time periods on haematological parameters and phagocytic activity in the blood of koi carp. The results showed that low concentrations of chloramphenicol (2 mg L\(^{-1}\) or 5 mg L\(^{-1}\) for 10 days) or oxytetracycline (20 mg L\(^{-1}\) for 3 days or 10 days) had no or mild effects on the parameters studied. However, treatment of fish with 10 mg L\(^{-1}\) chloramphenicol for 10 days depressed haematocrit, erythrocyte counts and mean corpuscular volume leading to anaemia. It also induced leucocytosis coupled with neutrophilia, thrombocytosis and lymphocytosis. The phagocytic index of the fish was also enhanced significantly with respect to the controls. The treatment of fish with 100 mg L\(^{-1}\) oxytetracycline induced leucopenia coupled with neutropenia. Phagocytic index of these fish was significantly depressed compared to the controls. Results revealed that precautions should be taken when high concentrations of chloramphenicol or oxytetracycline are used in koi carp culture especially for long term treatments.
REFERENCES


