In vitro study of oxytetracycline adsorption on activated charcoal

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IN VITRO STUDY OF OXYTETRACYCLINE ADSORPTION ON ACTIVATED CHARCOAL

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ABSTRACT

In vitro adsorption experiments simulating pH in gastric environment and using Langmuir isotherm, showed that 408 mg of oxytetracycline was adsorbed per gram of activated charcoal. Langmuir isotherm fitted adsorption data better than a Freundlich isotherm. Freundlich isotherm showed a specific adsorption capacity of 518 mg/g for activated charcoal. Both isotherm parameters indicated a strong oxytetracycline adsorption on activated charcoal in terms of quantity and binding strength. The results demonstrate that the concomitant use of oxytetracycline and activated charcoal should be avoided.

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INTRODUCTION

Products of oxytetracycline hydrochloride (OTC) have many commercial names (Terramycin-Pfizer, Oxyvet-Veterin) and significant advantages such as wide antimicrobial spectrum, long half-life (>6 hours) and ability of administration. Such advantages suggest OTC use as an appropriate therapeutic and chemoprophylactic agent for veterinary purposes. OTC containing formulations are usually food or water additives, although injectional forms are routinely used in cases of acute microbial infections.

Activated charcoal (AC) is the main component of many pharmaceutical formulations like Carbomix or Norit (Norit, US) and is characterised by high adsorptive properties. Its extremely high surface area ranges from 400-2.000 m$^2$/g (supercoals > 3.500 m$^2$/g) (Cooney, 1995) and makes it a highly efficient non-specific antidote. Many in vitro (Roivas and Neuvonen, 1992; Cooney and Thomason, 1997; Atta-Politou et al., 1996) or in vivo (Olkkola and Neuvonen, 1984; Minton and Henry, 1995; Laine et al., 1996) experiments have been performed, all indicating that the use of AC results in a reduction in the concentration of many drugs or toxic substances in the stomach and subsequently in the systemic blood circulation.

The use of AC in veterinary practice is recommended mainly in cases of acute mycotoxicosis and rarely when food is suspected for mycotoxin contamination (Poppenga et al., 1987; Dalvi and McGowan, 1984). AC is also used against many acute microbial diarrheal diseases (e.g. Clostridium perfrigens) acting as an adsorbent against microbial toxins (Mousouras, 1996). The non specific activity of AC could possibly cause undesirable effects associated with AC adsorption of vitamins, inorganic substances (metals) and antibiotics. Such an action could cause reduced antimicrobial action and reduced vitamin absorption etc. The adsorption of tetracycline antibiotics from charcoal and other adsorptive clays has not been studied extensively (Khalil et al., 1976; Browne et al., 1980).

The objective of this study was to evaluate in vitro the absorption of oxytetracycline on activated charcoal. The results of the present in vitro study
showed OTC was highly adsorbed on activated charcoal. The data demonstrated that concomitant administration of these two formulations should be avoided or performed with care.

MATERIALS AND METHODS

Materials

Oxytetracycline hydrochloride (purity > 99%) was kindly offered by Veterin Hellas S.A. Athens, Greece. Activated charcoal was the main ingredient of Carbomix (81.3% g/g) (Norit, US). Water was of HPLC grade (Merck, Darmestaad, Germany) while all other chemicals were of analytical grade.

Instrumentation

A Jasco 7800 UV-VIS spectrophotometer connected with a Jasco Ptl-396 printer (Jasco, Japan) were used for measuring the free (bulk) concentration of OTC solutions. A 74578 Bioblock scientific agitator (Bioblock Scientific, France), a P107 pH-meter (Consort, England) and an Econospin centrifugator (Sorvall Instruments) were used for agitating, pH-adjusting and cleaning OTC solutions.

Preparation of Stock Solutions and Standards.

Initial stock solutions were prepared by dissolving 0.3 g of OTC in 100 ml of aqueous hydrochloric solution and adjusting the pH to 2.35. Fifteen OTC solutions of concentrations ranging from 1.7 to 51.9 µg/ml were measured spectrophotometrically at 351 nm, where the highest absorbance peak of OTC was detected. The calibration process was repeated five times and the mean slope of the regression lines was estimated at 36.499 µg/ml, while mean variation coefficient of slopes did not exceed 2.2%. The r²-coefficients of linear fittings ranged from 0.9971-0.994.

Adsorption Kinetics and Batch Adsorption Experiments

Adsorption kinetics were studied using seven OTC solutions of concentration 1.846 mg/ml- prepared by diluting the initial stock solution with hydrochloric solution -pH=2.35. After the addition of 0.100 g of Carbomix (0.082
(g of AC) to the OTC solution the resulting mixtures were agitated (400 rpm) at room temperature for 15, 20, 45, 60, 90, 120 and 150 min. A solution was selected and filtered at each time interval. Traces of charcoal were cleaned after centrifugation at 3000 rpm for 20 min and the supernatant was delivered for spectrophotometric measurements. When necessary, solutions were diluted up to 11 times so that the absorbance fell within Beer’s law region. The above experimental process was repeated 3 times.

Ten OTC solutions of concentrations ranging from 0.923 to 3.000 mg/ml were prepared for batch adsorption experiments, by diluting the initial stock solution. OTC solutions were agitated for 2.0 h and measurement of free (bulk) concentration of OTC was made according to the methodology described for adsorption kinetics.

Mathematical Modeling of Adsorption

The linear form of Langmuir isotherm (1) and the double-log form of Freundlich isotherm (2) used to simulate the adsorption data are presented below.

\[
\begin{align*}
C_{\text{free}} \times ml/q_{\text{ads}} &= C_{\text{free}}/N + 1/(NK) \\
\log(q_{\text{ads}}/m) &= \log(k) + n \times \log(C_{\text{free}})
\end{align*}
\]

Langmuir isotherm supposed that the mechanism of adsorption has standard energy of binding, adsorption sites can not exceed a monolayer coverage, and that no interactions were specified between adsorbent molecules (Clark, 1970). Freundlich isotherm is an empirical formula but it is widely used because of its great interpretation capacity of adsorption data.

Variables used in the priormentioned isotherm are: \(C_{\text{free}}\) (mg/ml) (the free concentration of OTC in solution or the concentration of OTC remaining in solution when adsorption equilibrium is reached), \(q_{\text{ads}}\) (mg) (the quantity of OTC adsorbed), \(m\) (g) (the mass of activated charcoal used).

Four constants are presented in these isotherms: the \(N\) constant of Langmuir isotherm (mg/g), usually called Maximum Adsorption Capacity (MAC),
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FIGURE 1
Adsorption kinetics data for oxytetracycline hydrochloride on activated charcoal

which is a measure of binding capacity of activated charcoal, the $K$ constant (lt/g) which is the chemical equilibrium constant of the reaction between adsorbate-adsorbent, the $k$ constant (mg/g) which corresponds to the $N$ constant of Langmuir isotherm and $n$ which is a dimensionless constant dependent on experimental conditions like quantity of adsorbent and concentration of adsorbent etc.

Excel for Windows 95 was used for statistical and graphical presentation of data.

RESULTS AND DISCUSSION

Figure 1 depicts the obtained data from adsorption kinetics within a period of 2.5 h. More than 85% of the initial quantity of OTC has been adsorbed 45-50 min after the start of adsorption experiments. Adsorption equilibrium is shown to be reached at time greater than 90 minutes (Fig. 1).
FIGURE 2
Typical experimental data for oxytetracycline hydrochloride adsorption on activated charcoal fitted by Langmuir isotherm

In Figure 2 typical adsorption data and Langmuir adsorption isotherm of OTC on AC are shown. Slope of the plot estimates the reciprocal of \( N \) constant while \( K \) constant can be easily calculated by combining the estimated \( N \) values and the intercept of the plot. A much better fitting seems to be achieved using the Langmuir isotherm, in comparison to Freundlich isotherm shown in Figure 3. The \( k \) value can be easily calculated as a tenth power of the estimated \( \log(k) \)’s. The \( n \) values are easily estimated as the slope of regression plots (Figs. 2 and 3).

It is derived from Table 1 that the mean value ± standard deviation of \( N \), between runs, (SDr) is estimated at 408.0 ± 14.0 mg/g while chemical equilibrium constant \( K \) is 131.8 ± 18.5 lt/g, using Langmuir isotherm. Mean values and standard deviation (SD) of \( k \) constant derived from Freundlich isotherm (548.5 ± 48.0, 509.3 ± 36.7, 495.0 ± 37.7 mg/g) are also shown in Table 1.

In Table 2 suggested OTC daily doses in animal food, or water doses are shown. These doses are suggested for therapy of microbial infections and are dependent on the type of animal, and the type of formulation.
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FIGURE 3
Typical experimental data for oxytetracycline hydrochloride adsorption on activated charcoal fitted by Freundlich isotherm.

TABLE 1
Adsorption Constants for Oxytetracycline Hydrochloride Adsorption on Activated Charcoal Simulated by Langmuir and Freundlich Isotherms

<table>
<thead>
<tr>
<th>Run</th>
<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N \pm SD$ (mg/g)</td>
<td>$K \pm SD$ (L/g)</td>
</tr>
<tr>
<td>1</td>
<td>424.1 ± 9.6</td>
<td>151.5 ± 65.4</td>
</tr>
<tr>
<td>2</td>
<td>401.7 ± 7.8</td>
<td>129.2 ± 59.2</td>
</tr>
<tr>
<td>3</td>
<td>398.2 ± 4.3</td>
<td>114.7 ± 28.7</td>
</tr>
</tbody>
</table>

SD: Standard deviation of coefficient estimated by linear regression
SD*: Standard deviation between runs (3 runs)
### Table 2

**Suggested Dosages of OTC for Veterinary Purposes Using Various OTC Formulations**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Oxytetracycline dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per kg of animal weight</td>
</tr>
<tr>
<td>Sheep</td>
<td>0.5 - 0.75 g/25 kg</td>
</tr>
<tr>
<td>Swine</td>
<td>0.5 - 1.5 g/50 kg</td>
</tr>
<tr>
<td>Birds/hens</td>
<td>1.0 - 2.0 g/100 kg</td>
</tr>
<tr>
<td>Calve</td>
<td>1.0 - 2.0 g/100 kg</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.5 - 5 g/10 kg</td>
</tr>
</tbody>
</table>

+ Dosages are estimated to net weight of OTC used in formulations

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**Adsorption**

To avoid errors in calculating adsorption constants like specific or maximum adsorption capacity Freundlich or Langmuir isotherm should be used when an adsorption equilibrium is reached. According to adsorption kinetic results shown in Figure 1 the experimental time of 2 hours is appropriate (equilibrium time ~90 min).

As it is shown in Figures 2 and 3, Langmuir isotherm fits adsorption data better than Freundlich isotherm. This can also be seen from the results shown in Table 1, where $r^2$ values ranged from 0.9959 to 0.9990 for Langmuir isotherm, and $r^2 \approx 0.900$ for Freundlich isotherm. These results are in agreement with results from other studies, were the Langmuir isotherm is used -mainly in aqueous solutions- (Kanisto and Neuvonen, 1984; Tcuchiya and Levy, 1972; Neuvonen et al., 1984) and are in disagreement with results from other studies (Cooney and Thomason, 1997) were the Freundlich isotherm is used.

Although Freundlich isotherm describes only 90% of the variance estimated values of the $n$ constant, they are found to be similar between runs (range 0.1701-0.1742). Such values should be expected because $n$ constant characterises the interactions between adsorbent-adsorbate. Changes of $n$ should be expected when experimental conditions (temperature, type of adsorbent or
adsorbate, pH etc) are different. The value of $k$ constant according to Freundlich isotherm was estimated higher than the corresponding constant $N$ of Langmuir isotherm. The $K$ -values of Langmuir isotherm show a strong binding capacity between adsorbent - adsorbate molecules. In conclusion, both isotherms indicated a strong oxytetracycline adsorption on activated charcoal in terms of quantity and binding strength.

**OTC - AC Incompatibility for Concomitant Use**

Food or water additives containing OTC are used daily for chemoprophylaxis against microbial infections and suggested doses are presented in Table 2. Experiments refer to the use of OTC usually in combination with other formulations against acute mycotoxicosis (Wilson et al., 1974). Experimental aflatoxicosis and therapy including the use of OTC and AC have been described (Hatch et al., 1982).

Dose of AC in cases of acute mycotoxicosis ranged from 2 to 6.6 g per kg body weight. Dose of 0.1-1 g per kg of animal food led to the disappearance or decrease of symptoms of chronic mycotoxicosis (liver lesions, increase of hepatic enzyme levels in blood etc) (Ramos et al., 1996). Doses of 20-120 mg/kg of body weight were used for infections caused by microbial toxins (Mousouras, 1996). Experimental data of *in vitro* or *in vivo* experiments showed that significant amounts of aflatoxins B1, B2, G1, G2, and ochratoxin A were adsorbed using small quantities of AC (Ramos et al., 1996).

Levels of OTC and AC are comparable to those used in our experiments (OTC initial quantities 12-39 mg, AC initial quantity 0.1 g). Consideration was taken in that the used formulations of activated charcoal in veterinary usually have greater adsorption capacities than Carbomix®.

Estimated constants showed that OTC is highly and strongly adsorbed on AC. In cases where AC is given as an antidote, formulations containing OTC should be administered with care.
CONCLUSION

OTC was strongly adsorbed on activated charcoal. Concomitant administration of OTC and AC should be avoided or should be performed with care when needed. In cases where toxic symptoms are presented and AC is given, formulations of OTC be should administered through other means, such as intramuscularly or intravenously.

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REFERENCES


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