Pharmacokinetic and pharmacodynamic properties of metomidate in turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*)

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Metomidate was administered to halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) intravenously at a dose of 3 mg/kg bodyweight, as a bath treatment at a dose of 9 mg/L water for 5 min to study the disposition of metomidate, and as bath treatment (9 mg/L) for 10 min to study the absorption and effect of metomidate on respiration and balance/motor control. Additionally, turbot were given metomidate orally at a dose of 7 mg/kg. The studies were performed in seawater at a temperature of 10.3 ± 0.4 °C (halibut) and 18.0 ± 0.3 °C (turbot). Pharmacokinetic modeling of the data showed that metomidate had shorter elimination half-life and higher plasma concentrations in turbot compared with halibut, both species displaying a rapid uptake, distribution and excretion. Following intravenous administration, the volumes of distribution at steady state ($V_{d(ss)}$) were 0.21 L/kg (halibut) and 0.44 L/kg (turbot). Plasma clearances (*Cl*) were 0.099 L/h·kg in halibut and 0.26 L/h·kg in turbot and the elimination half-lives ($t_{½}\lambda_z$) were calculated to be 5.8 h and 2.2 h in halibut and turbot, respectively. Mean residence times (MRT) were 2.2 h in halibut and 1.7 h in turbot. Following oral administration, the $t_{½}\lambda_z$ was 3.5 h in turbot. The maximum plasma concentration (C_{max}) was 7.8 mg/L in turbot 1 h after administration. The oral bioavailability (F) was calculated to 100% in turbot. Following 5 min bath the maximum plasma concentrations (C_{max}), which were observed immediately after end of the bath, were 9.5 mg/L and 13.3 mg/L in halibut and turbot, respectively. Metomidate rapidly immobilized the fish, with respiratory depression, reduced heart rate, and loss of balance/motor control within 1 min (mean). Recovery was slow, with resumed balance/motor control after 26.4 min. Opercular respiration movements were resumed more rapidly with a recorded mean of 1.7 min. Oral administration was demonstrated to be a way of immobilizing fish, for example in large aquariums, without exposing them to unwanted stress.

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INTRODUCTION

Turbot (*Scophthalmus maximus*) and halibut (*Hippoglossus hippoglossus*) are two important nonsalmonid fish species now emerging in the European aquaculture industry. The production in 2000 was approximately 5000 tons of turbot, mainly produced in Spain. Halibut is a marine cold-water species (8–10 °C) currently being developed for aquaculture. The annual production of halibut in Norway and Iceland is currently approximately 750 000 fry, which will produce approximately 2500 tons when slaughtered.

Immobilizing/anesthetic agents in marine fish species have mainly been utilized extrapolating the pharmacodynamic and pharmacokinetic data known from salmonids (Atlantic salmon, *Salmo salar* and rainbow trout, *Oncorhynchus mykiss*). Knowledge of the pharmacokinetic and pharmacodynamic properties of drugs used in the actual species is vital for their correct application. The use of benzocaine, the most commonly used anesthetic agent in Norwegian salmon aquaculture, to anesthetize cod (*Gadus morhua*) has several times caused mortalities when used at the same dose as that used for salmon (Mattson & Riple, 1989).

In commercial fish farming and in scientific studies, certain procedures often require the use of anesthesia or immobilization. One immobilizing/anesthetic agent used in flatfish and other

marine species is metomidate. This moderately lipophilic carboxylated imidazole with the properties of a weak base (pKa approximately 4.2, partition coefficient oil/water (pK_{ow}) 2.46) induces deep sleep without removing the reaction to severe pain (Reneman & Janssen, 1977; on etomidate), and should therefore not be used as the only drug for painful procedures.

In some marine species, metomidate seems to have a wider therapeutic range compared with benzocaine and metacaine (Mattson & Riple, 1989; Malmstrom *et al.*, 1993).

Until 1997, metomidate was commonly used in anesthesia for swine in Europe, but is no longer used because no maximum residue limit (MRL) has been established within the European Union yet (Ungemach *et al.*, 1997). Metomidate for anesthesia of fish is still available in some countries. The aim of this study was to investigate pharmacokinetic properties and the effect (pharmacodynamic properties) of metomidate in halibut and turbot.

MATERIALS AND METHODS

Formulation of test substance

Metomidate (Hypnodil™; 50 mg/mL metomidate HCl) was obtained from Janssen Pharmaceutical, Beerse, Belgium. The solution for intravenous (i.v.) administration was prepared by diluting metomidate in sterile saline (0.9%) to a concentration of 3 mg/mL. Metomidate for oral administration was mixed into a 4:3 emulsion of minced ordinary fish feed: cod liver oil to a concentration of 7 mg/mL. For bath administration, the formulation Hypnodil™ was diluted according to label. The measured concentrations in the i.v.-solution, the oral formulation and in the bath solutions confirmed with a high performance liquid chromatography (HPLC)-assay, differed slightly from the intended nominal values. In the pharmacokinetic calculations, the concentrations measured in each formulation were used to calculate the dose; i.v. dose of 3.1 mg/L (turbot) and 2.6 mg/L (halibut); oral dose of 7.0 mg/kg (turbot); bath treatments (8.9 mg/L).

Test facilities and test fish

The study was conducted at Stolt Seafarm, Øye, Norway. The fish were raised at the farm, and were held in fiberglass tanks of 1000 L supplied with running seawater with a salinity of about 3%. The turbot (*S. maximus*) were approximately 8 months old and weighed 228 (70) g [mean (SD)]. They were held in running seawater at the optimal growth temperature of 18.0 ± 0.5 °C. The halibut (*H. hippoglossus*) were approximately 1 year old and weighed 166 (47) g [mean (SD)] and were held in running seawater at the optimal growth temperature of 10.3 ± 0.5 °C.

Intravenous administration

The fish being administered metomidate intravenously were allocated into groups of six, which were administered metomidate individually at a dose of 3 mg/kg into the caudal vein. The injection was performed with the fish positioned on a damp cloth, with the bottom side down during weighing and injection. The metomidate solution was slowly injected using a 1-mL disposable syringe and a 0.5 × 25 mm needle (Terumo, Leuven, Belgium). The position of the needle was confirmed by aspiration of blood before, during and after the injection. Fish in which the needle dislocated during the injection were discarded and replaced.

Oral administration

The turbot which were given the drug orally, were allocated into small groups of six, and administered metomidate individually at a dose of 7 mg/kg through a stomach tube (Martinsen *et al.*, 1993). Halibut were not administered metomidate orally, as the number of individuals available was limited.

Bath administrations

The bath administrations in the disposition and absorption study were carried out in two 1000 L fiberglass tanks with 100 L static aerated seawater. Metomidate was added to the seawater to a final concentration of 9 mg/L. The fish in the disposition study were allocated into groups of six and kept in the metomidate bath solution for 5 min The fish in the absorption study, also allocated into groups of six, were kept in the bath solution from 1 to 10 min Thereafter they were transferred to flow-through water tanks.

The pharmacodynamic study, dealing with the effect on respiration and heart rate, was carried out in 10 L glass aquariums with 5 L static aerated seawater. Metomidate was added to the seawater to a final concentration of 9 mg/L and a total of 17 fish were kept in the metomidate bath solution for 10 min.

Sampling

Blood samples (100 μL) were collected at 0.33, 1, 3, 7, 12, 18, 24, 48, 96 and 168 h following i.v. and oral administration (six fish at each time point). The blood samples were collected caudal to the injection site in the intravenously administered group by venipuncture, using a 0.5 × 25 mm needle and 1 mL syringe.

In the bath exposed groups, blood samples (100 μL) were collected at 0, 1, 3, 7, 12, 18, 24, 48, 96 and 168 h after administration from six fish at each time point. In the absorption study, though, blood were only collected at 1, 2, 3, 5, 7 and 10 min during the exposure time from six fish at each time point.

All the blood samples were frozen at −80 °C until analyzed.

No mortalities were recorded in the experimental fish. The sampled blood volume (100 μL) comprised less than 2% (approximately) of total blood volume.

Analytical procedures

To the plasma samples (40 μL) were added propoxate (40 μL = 80 ng propoxate in methanol-water, 1 + 2) as an internal standard. After mixing (10 sec) in a centrifuge tube,

50 μL 40 mM sodiumacetate buffer, pH 5.0 was added and the solution mixed for 10 sec. A quantity of 3 mL n-pentane was added and metomidate and propoxate was extracted into the organic phase, using a vertical rotating carousel (2.1 ***g***, 10 min). After centrifugation (3000 ***g***, 10 min, 4 °C), n-pentane was transferred to a conical centrifugation tube. Evaporation of n-pentane was carried out under a stream of nitrogen at 30 °C for about 10 min. The residue was dissolved in 100 μL mobile phase and mixed for 1.5 min. After centrifugation (3000 ***g***, 5 min, 10 °C), the extract was transferred to a vial, for injection on the HPLC system.

Chromatography

Metomidate was separated from other plasma components on an Alltima C18, 150 × 4.6 mm, 5 μm analytic column (Alltech Deerfield, MA) with an Alltima C-18, 5 μm, 7.5 × 4.6 mm precolumn (Alltech). The system was operated at room temperature with a mobile phase consisting of acetonitrile and 14 mM sodium acetate in water [42:58 (v:v)]. The pH was adjusted in the mobile phase to 5.8. The flow was 1.2 mL/min, and the injection volume was 40 μL. The compound was detected on a UV detector at 250 nm. Data sampling time was 20 min with a cycle time of 61 min to avoid interferences. The lower limit of quantitation of the method was 15 μg/L, and it was linear over a tested range of 125–12 500 μg/L. The linear correlation coefficient was 0.999. The linearity of the calibration curve was also tested on a residual plot, revealing no bias. The recovery of metomidate and propoxate in calibration plasma ($n = 6$) was 96.2% and 81.0%, respectively.

Pharmacokinetic analysis

Pharmacokinetic evaluation was performed using the computer program WinNonlin, version 1.1 (Statistical consultants Inc., Lexington, KY, USA). Standard pharmacokinetic parameters were calculated according to a noncompartment model. In the i.v.-group, the intercept with the *y*-axis was calculated by back-extrapolation of the curve, using the first two data points. In all groups, the curve was extrapolated to infinity using the λ_z calculated from the depletion data, in a linear regression analysis using the last three or more time points according to the algorithm of Dunne (1985).

The bioavailability of the oral preparation was calculated by comparing the area under the concentration time curve $(AUC)_{i.v.}$ (0–∞) and AUC_{PO} (0–∞) corrected for dose.

Recording of respiration frequency and balance/motor control in turbot

The effect of metomidate on respiration and balance/motor control was recorded in fish from the same group as the kinetic study. Respiration frequencies were visually observed and manually plotted using a data-plotter (AAC2, INTAB), and were recorded in EasyView 4.0 (INTAB, Stenkullen, Sweden). The respiration frequency curves were calculated as respiration frequency per minute. The mean curve was made using the frequencies registered in every 10-sec interval for all individuals. The time interval following immersion in the bath at which the fish could be turned bottom up without turning back was recorded, and the time interval following transfer to unmedicated water at which it spontaneously turned back was recorded as recovery time.

The heart rate was registered in the same way as the respiration frequency, in nonpigmented turbot.

RESULTS

The estimated $t_{½}\lambda_z$ was 5.8 h in halibut and 2.2 h in turbot. The observed $V_{d(ss)}$ was 0.21 L/kg in halibut and 0.44 L/kg in turbot, and the total body Cl_T was 0.099 L/h·kg and 0.26 L/h·kg for halibut and turbot, respectively. Mean residence time was 2.2 h in halibut and 1.7 h in turbot.

Maximum plasma concentration (C_{max}) after oral administration was 7.8 mg/L in turbot 1 h after administration (T_{max}). The $t_{½}\lambda_z$ after oral administration was calculated to be 3.5 h and the bioavailability (F) to be 100% in turbot.

After bath-administration (9 mg/L for 5 min), C_{max} of 9.5 mg/L in halibut and 13.3 mg/L in turbot was detected at 0 h (T_{max}) after administration. The pharmacokinetic

Table 1. Pharmacokinetic parameters in halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) held in running seawater at 10 °C and 18 °C, respectively, following a single dose of 3 mg metomidate/kg bodyweight intravenously, 7 mg/kg orally or 9 mg metomidate/L in seawater bath for 2 h. The parameters were calculated using a noncompartmental model

	$t_{½}\lambda_z$ (h)	*Cl* (L/h·kg)	$V_{d(ss)}$ (L/kg)	C_{max} (mg/L)	T_{max} (h)	F (%)
i.v. 3 mg/kg						
Turbot	2.2	0.26	0.44			
Halibut	5.8	0.099	0.21			
Bath 9 mg/L 5 min						
Turbot				13.3		
Halibut				9.5		
Oral 7 mg/kg						
Turbot	3.5			7.8	1	100

AUC 0–∞: area under plasma concentration–time curve extrapolated to infinity; $t_{½}\lambda_z$: elimination half-life during the elimination phase; MRT: mean residence time; $V_{d(ss)}$: volumes of distribution at steady state; *Cl*: plasma clearance; C_{max}: maximum plasma concentration; T_{max}: time of peak plasma concentration; F: bioavailability.

parameters are listed in Table 1, and the plasma concentration vs. time curves for metomidate in halibut and turbot are shown in Figs 1 and 2, respectively.

Both turbot and halibut were rapidly hypnotized by metomidate (Table 2 and Fig. 4). After 37 sec (mean), the turbot was calm, with no swimming movements. After approximately 1 min (mean) the turbot did not turn back when turned bottom up. The respiration in turbot and halibut was depressed by metomidate. In turbot, normal respiration movements ceased after approximately 3.4 min. Also the frequency of respiration movements was depressed as shown in Fig. 4. The heart rate was also depressed by metomidate as shown in Fig. 5. The recovery was slow in both turbot and halibut. An average time of 24 min was recorded before the turbot turned back to normal body position. Normal respiration movements resumed more rapidly; 1.7 min (mean) in turbot. The respiration rate seemed to recover more slowly (Fig. 4). After oral administration, the induction of anesthesia was relatively rapid, and the turbot could be turned bottom up without turning back after 3.2 min (mean). Recovery was slow and comparable with bath exposure, and a mean of 26.8 min was recorded before the turbot turned back.

DISCUSSION

In pharmacokinetic studies in mammals, blood samples are collected at different time points after drug administration from each individual throughout the whole study period. In the current study, only small experimental fish were available (halibut 166 g, turbot 228 g), and frequent blood sampling from each fish was considered impossible. Therefore we chose a design where we collected blood samples from each fish two times (single individual – single sample design; Horsberg, 1994).

Metomidate is closely related to etomidate ((R)-(+)-ethyl-1-(1-phenylethyl)-1H-imidazole-5-carboxylate), and has comparable physiochemical and pharmacological properties. Etomidate is a more potent, more lipophilic hypnotic compound. Because pharmacokinetic data for metomidate are not available, we have compared some of our results with the data reported from studies with etomidate.

The doses of 3 mg/kg for i.v. administration and 7 mg/kg for oral administration were chosen after a preliminary study. The dose of 9 mg/L given in the bath treatment was chosen in accordance with previous reported studies on metomidate

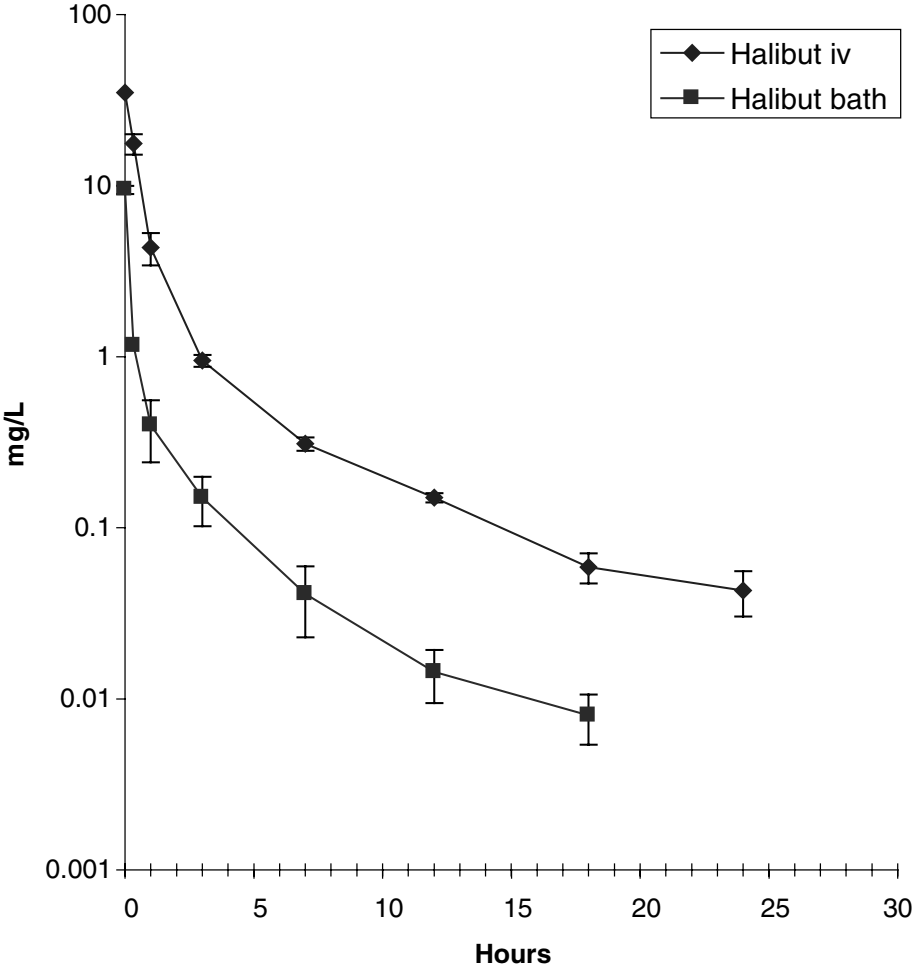

Fig. 1. Plasma concentration profiles (mean ± SEM) of metomidate in halibut ($n = 6$) following a single dose (3 mg/kg) administered intravenously (i.v.), and a single dose (9 mg/L) administered as a bath treatment. SD at each sampling time is given in Table 4.

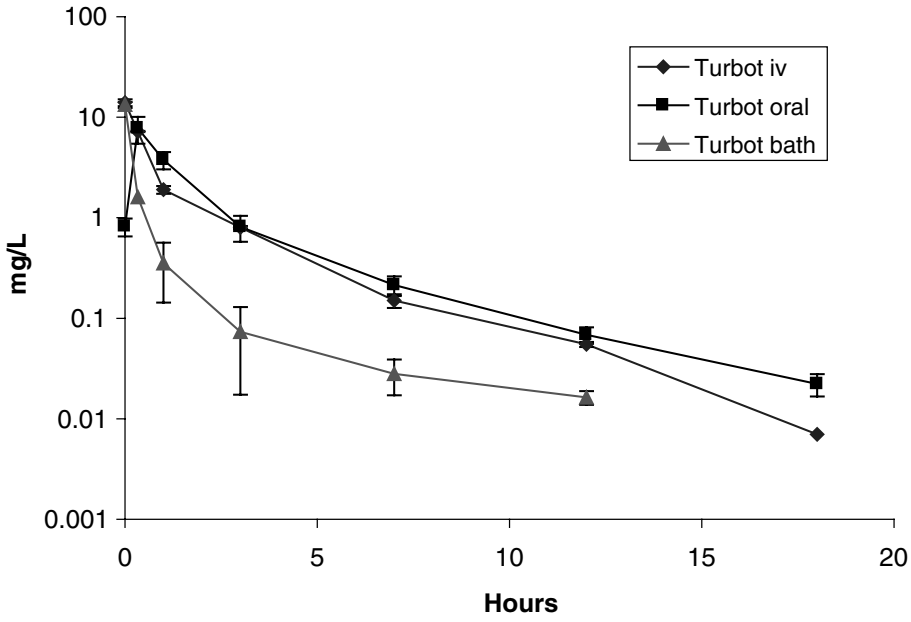

Fig. 2. Plasma concentration profiles (mean ± SEM) of metomidate in turbot ($n = 6$) following a dose (single 3 mg/kg) administered intravenously (i.v.), a single dose of 7 mg/kg administered orally, and a single dose (9 mg/L) administered as a bath. SD at each sampling time is given in Table 4.

(Mattson & Riple, 1989; Malmstrom *et al.*, 1993; Olsen *et al.*, 1995; Grottum *et al.*, 1998).

In the oral test formulation, the amount of cod liver oil was adjusted to make the suspension suitable for administration through a stomach tube.

A noncompartment model was used in the pharmacokinetic calculations. Other ways of modeling the data were also tested. The data from the i.v. administered group in halibut could be interpreted using a three compartmental modell, based on the minimal Akaike's information criterion estimation (Yamaoka *et al.*, 1978) with weighing of the data in the elimination phase (weight −1). In this model the $t_{½}\gamma$ was estimated to be 6.7 h. In turbot, the data could be interpreted using a two-compartment modell (unweighted data). The estimate of $t_{½}\beta$ was 2.0 h. The $t_{½}\lambda_z$, $V_{d(ss)}$ and *Cl* were nearly the same after modeling with noncompartment and the 2- and 3-compartment models.

As the water temperature may influence the drug disposition in fish significantly, turbot and halibut were kept at their optimal growth temperatures to ease comparison. Differences in temperatures should always be considered when comparing pharmacokinetic and pharmacodynamic parameters in fish. Stehly *et al.* (1998) reported increased clearance with increased temperature, but no effect on elimination half-life, for the anesthetic agent benzocaine in rainbow trout (*Oncorhynchus mykiss*).

There are to our knowledge no pharmacokinetic studies on metomidate in fish. Our results from the i.v.-administered group showed that metomidate had similar pharmacokinetic properties in halibut and turbot. Turbot, though, displayed shorter elimination half-life and higher plasma concentrations compared with halibut. The $V_{d(ss)}$ was estimated to be 0.21 L/kg (halibut) and 0.44 L/kg (turbot). Despite the relatively small $V_{d(ss)}$, the drug is probably, extensively distributed to well-perfused tissues like the brain. Several studies on the closely related hypnotic drug etomidate have shown that well perfused tissues (like the brain) are in true equilibrium with plasma in mammals (Lewi *et al.*, 1976; Meuldermans & Heykants, 1976; Reneman & Janssen, 1977). The volumes of distribution observed in our

Table 2. Respiration and balance/motor control in turbot (*Scophthalmus maximus*) following metomidate administrated as a bath (9 mg/L for 10 min) or orally (7 mg/kg). Time in minutes

	Calmed	Loss of righting reflex	Ceased opercular respiratory movement	Start opercular respiratory movement (Following transfer to unmedicated water)	Turned back (righting reflex) (Following transfer to unmedicated water)
Bath					
Mean	0.6	1	3.4	1.7	26.4
SD	0.23	0.53	0.60	0.60	5.3
Range	0.28–1.15	0.28–1.83	2.16–4.50	0.63–2.98	19.0–38.0
Oral					
Mean	2.8				27.0
SD	0.92				11.12
Range	2.5–5.0				11.0–35.0

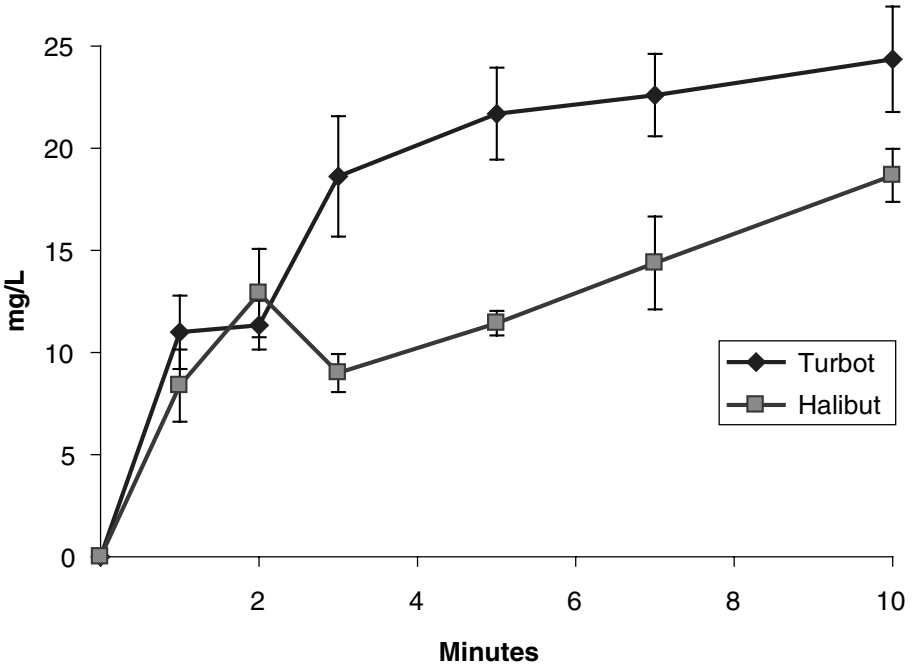

Fig. 3. Plasma concentration profiles (mean ± SEM) of metomidate in halibut ($n = 6$) and turbot ($n = 6$) during bath treatment (9 mg/L metomidate for 10 min).

study are small compared with studies on etomidate in mammals ($V_d = 1.6$ L/kg in rat (Lewi *et al.*, 1976); 2.05 L/kg in dog (Zhang *et al.*, 1998); 4.49 L/kg in humans (van Hamme *et al.*, 1978). This may be as a result of differences in solubility in the lipid containing compartments.

Bergström *et al.*, 1998) studied tissue distribution of ^{11}C-metomidate and ^{11}C-etomidate in different abdominal organs of rat and pig (frozen section autoradiography) and monkey (PET-imaging). The tissue distribution of ^{11}C-metomidate and ^{11}C-etomidate were similar, with the highest concentrations seen in adrenals and liver. Lewi *et al.* (1976) reported brain to be the tissue containing most (relatively) etomidate in the rat (1.37% of dose per gram).

Total body *Cl* were 0.099 L/h·kg in halibut and 0.260 L/h·kg in turbot. The lower *Cl* of halibut influences its terminal elimination half-life, which is calculated to be about twice as long as in turbot at the temperatures used. Van Hamme *et al.* (1978) reported *Cl* for etomidate in humans to be 0.699 L/h·kg and Zhang *et al.* (1998) reported *Cl* for etomidate in dog to be of 0.017 L/h·kg. Recovery times are reported to be longer for metomidate than for benzocaine and the related drug MS-222 (Mattson & Riple, 1989; Iwama *et al.*, 1989). The lower *Cl* of metomidate compared with benzocaine (0.75 L/h·kg in rainbow trout; Meinertz *et al.*, 1996) may explain this difference. Both metomidate in cod (Mattson & Riple, 1989) and etomidate in humans (Reneman & Janssen, 1977) have been shown to have relatively shorter recovery times when administered high doses compared with low doses. This may be because of the fact that lower doses necessitate longer exposure times, and hence more of the compound will be distributed in the body.

The $t_{½}\lambda_z$ after i.v.-administration were relatively short, and estimated to be 5.8 and 2.2 h for halibut and turbot, respectively. This is similar to reported $t_{½}\lambda_z$ of etomidate in dog (01.44 h; Zhang *et al.*, 1998), and humans (01.25 h; Reneman & Janssen, 1977; 04.59 h; van Hamme *et al.*, 1978; 3.3 h, Avram *et al.*, 1983).

Oral administration of metomidate was only studied in turbot. Maximal plasma concentration after oral administration was 7.8 mg/L, 1 h after administration, which shows a relatively rapid oral absorption.

The bioavailability was calculated to be approximately 100% and the $t_{½}\lambda_z$ was 3.5 h in turbot. This is a little longer than the $t_{½}\lambda_z$ calculated after i.v.-administration.

Little information is published on extra-vascular administration of immobilizing/anesthetic agents in fish. The rapid absorption and high bioavailability shown in turbot, together

Sample point (h)	Turbot i.v.	Turbot oral	Turbot bath	Halibut i.v.	Halibut bath
0.33	2.6	0.4	2.29	5.87	1.32
1	0.22	5.71	0.52	2.29	0.39
3	0.40	1.80	0.14	0.19	0.12
7	0.07	0.58	0.03	0.07	0.045
12	0.03	0.12	0.005	0.02	0.012
18	0.005	0.03	0.002	0.03	0.005

Table 3. SD of plasma concentrations (mg/L) of metomidate after administration of 3 mg/kg i.v., 7 mg/kg orally and 9 mg/L water for 10 min bath treatment

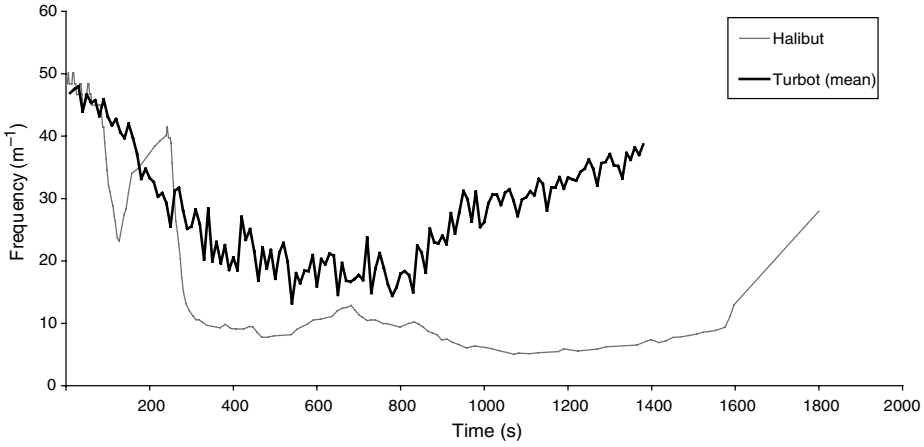

Fig. 4. Mean respiration frequency of turbot ($n = 14$) and halibut ($n = 1$) during and following bath exposure to 9 mg/L metomidate for 600 sec.

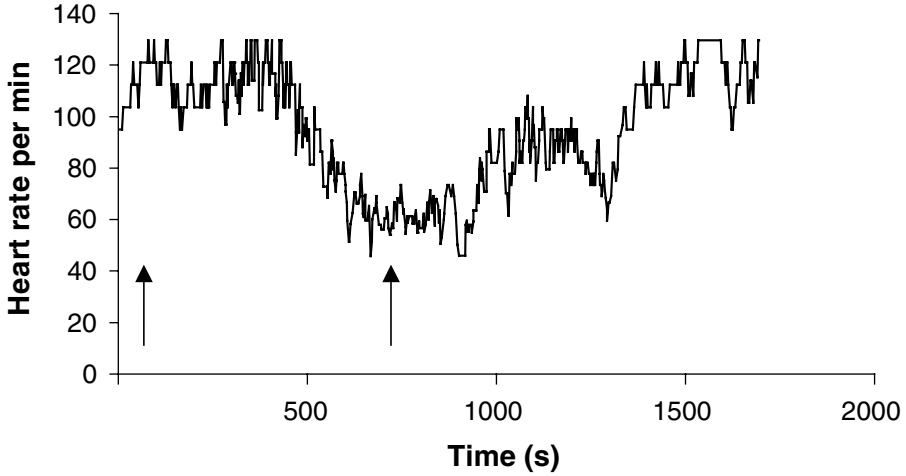

Fig. 5. Heart rate in turbot ($n = 2$) before, during and after bath administration in 10 mg/L metomidate.

with the relatively large therapeutic index (Mattson & Riple, 1989; Malmstrom *et al.*, 1993; Harvey *et al.*, 1988), indicates that oral administration of metomidate to immobilize fish may be possible. Capture, transport and handling of large, wild fish, e.g. in large aquariums are difficult operations. Netting followed by immersion in an anesthetic bath (e.g. MS-222, benzocaine), often lead to netting-trauma and delayed stress-related diseases and mortality (Tytler & Hawkins, 1981). Harvey *et al.* (1988) proposed the use of laser aimed underwater dart gun technology to capture and immobilize fish in large aquariums. Oral administration of immobilizing drugs would be a great advantage. The induction of anesthesia after oral administration was relatively rapid. Approximately 2.8 min (mean) post administration, the turbot had lost balance/motor control, and did not turn around when placed bottom up (Table 2). Recovery after oral administration was slow compared with the induction time. In 27 min (mean) the turbot managed to turn back into the normal position (Table 3).

The absorption process during bath exposure was studied in turbot and halibut administered metomidate at a dose of 9 mg/L water for up to 10 min. In halibut, the concentration vs. time curve (Fig. 3) rose sharply from 1 to 2 min, declined from 2 to 3 min and thereafter gradually increased. Turbot showed the same biphasic absorption as halibut (Fig. 3). This biphasic mode of absorption is probably because of distribution of metomidate from plasma to tissues during a period with reduced absorption as a result of cardiac and/or respiratory depression. The changes in the mode of respiration movements are even more obvious than the change in frequency. After 3.3 min (mean) exposure, the opercular movements ceased, and the jaws had stopped moving. Only the soft tissue under the tongue and the opercular edge moved a little. When normal respiratory movement ceases, the water flow passing the gills is greatly reduced or possibly stops. The gills in turbot and halibut are well sheltered behind the opercula and the almost closed mouth. The concentration of metomidate presented to the gills and its capillaries may therefore diminish because of the respiratory depression. As a result of technical problems, the heart rate could not be registered in the same fish as the respiration frequency. To get an indication of whether or not metomidate influence the heart rate, small nonpigmented fish, were used. In these fish the heart could be seen, and the heart rate recorded. Even though the fish was stressed because of handling, and a low number of fish used (2) the registrations indicated that metomidate significantly

decreased the heart rate. This has been demonstrated in rainbow trout for the closely related hypnotic drug etomidate (Fredricks *et al.*, 1993). Both respiratory volume (water flow) and cardiac output influences the absorption process across the gill (Hayton & Barron, 1990). Hayton *et al.* (1996) concluded that the benzocaine absorption rate in channel catfish (*Ictalurus punctatus*) was limited by the branchial blood flow, and that the water flow was not rate determining. They reported that the respiration decreased, but did not stop at any time during exposure.

The rapid onset of the effects of metomidate in our study is in accordance with results from previous studies on metomidate and etomidate in fish, reporting a rapid induction of anesthesia (Gilderhus & Marking, 1987; Mattson & Riple, 1989; Malmstrom *et al.*, 1993; Olsen *et al.*, 1995). There are several descriptions of anesthetic stages in fish (McFarland, 1959; Schoettger & Julin, 1967; Bell, 1987; Stoskopf, 1993). These descriptions all include loss of equilibrium and regulation of balance to evaluate the anesthetic stage. For flatfish like turbot and halibut, which normally rest on the bottom, these evaluation criteria do not function very well. Malmstrom *et al.* (1993) used the cessation of opercular respiration as an indication of 'anesthetic' stage and recovery. This makes comparison with studies in free-swimming fish difficult, as loss of and regained equilibrium is most often used to judge anesthetic stage and recovery time. Recovery in turbot and halibut were relatively slow. Our results show that opercular movements resume a long time (1.7 min) before normal respiration frequency or proper balance/motor regulation is regained (26 min). This is in accordance with the description of recovery stages by Bell (1987), dividing recovery into three stages. Stages 1 and 2 are characterized by start and frequency of opercular movements, whereas stage 3 is judged by regained equilibrium. Because of the limitations in the number of halibut available, respiration frequency was monitored in only one halibut. The depression of respiration frequency in this halibut seemed more severe, and the duration was longer compared with any of the monitored turbots. This is in agreement with the results from the pharmacokinetic study showing that halibut eliminates metomidate more slowly compared with turbot at the temperatures used in the present study.

No plasma concentrations have been related to the anesthetic effect of metomidate in fish. In the bath exposure absorption-study, an average plasma concentration slightly above 10 mg/L was found after 1 min. This time point corresponds to the time at which balance control had ceased in turbot. After 3–6 min exposure, the plasma concentration in turbot was approximately 18–22 mg/L. This time period corresponded to the time where respiration was significantly depressed. This is an indication of the plasma concentration that is necessary to obtain hypnotic effects in turbot.

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