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Alteration of Hepatic Microcirculation by Oxethazaine and Some Vasoconstrictors in the Perfused Rat Liver

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ABSTRACT. We previously reported that, in isolated perfused rat livers in a constant flow system, oxethazaine (OXZ) rapidly increased portal pressure (PP) accompanied by inhibition of oxygen uptake and the subsequent metabolic effects. In this study, hemodynamic changes were studied by using an indicator dilution technique and by microscopic observation of post-fixed liver samples stained with acridine orange or trapped fluorescence microspheres (FMSs). During the increase in PP induced by OXZ, the mean transit times of both red blood cells and azoalbumin were shortened markedly, and the vascular and extravascular albumin spaces decreased to 55 and 18% of the controls, respectively. With acridine orange, in the control livers, all the dye infused was taken up and the periportal zones were uniformly stained over all the liver sections, whereas in the OXZ-treated livers, about 30% of the dye drained out, and extensive staining was observed in the central portion of the liver mass, but the peripheral portions of the liver were much less stained. The staining was often localized around large portal vein branches and spread toward the hepatic veins. These changes were recoverable in the absence of OXZ. Distributions of 1-μm and 15-μm FMSs were likewise altered by OXZ. Thus, uneven perfusion may be the primary cause of decreased tissue spaces and also of the metabolic effects produced by OXZ. Endothelin 1 also produced OXZ-like changes, while U-46619 had lesser effects. The methodology used in this study may help delineate the hepatic perfusion disturbance caused by various vasoconstrictors. BIOCHEM PHARMACOL **53**;12: 1779–1787, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. portal pressure increase; hepatic perfusion disturbance; oxethazaine; vasoconstrictors; acridine orange staining

The liver is supplied by blood from the portal vein and hepatic artery, especially the former. These vessels repeatedly ramify and continue into the sinusoids, which perfuse the liver acinus, a functional unit of the liver, and finally the blood drains into the central vein toward the large hepatic vein. This hepatic circulation is under nervous and hormonal control and is affected by various physiological and pathological conditions [1]. In the isolated perfused liver, in which the liver is perfused through the portal vein, many substances are known to increase the PP†, including phenylephrine [2], ATP [3], UTP [3], the TXA_2 analogue U-46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $E_{2\alpha}$) [4, 5], leukotrienes C_4 and D_4 [5], ET [6, 7], and PAF [8, 9]. They often cause decreases in hepatic oxygen uptake and subsequent anoxic metabolic manifestations. However, to our knowledge, the mechanisms involved in the causal relationship between the hemodynamic and metabolic changes are not always clear.

OXZ, $HOCH_2CH_2\text{-}N[CH_2CON(CH_3)C(CH_3)_2CH_2\text{-}C_6H_5]_2$, is a topical anesthetic exhibiting potent action even at low pH, and has long been prescribed clinically for esophagitis, chronic gastritis, and peptic ulcers [10–12]. During a search for agents that prevent peroxidative liver damage, using the liver perfusion system, we happened to find that this anesthetic prevented liver injury induced by *t*-butyl hydroperoxide by reducing its hepatic uptake. Moreover, OXZ rapidly increased PP, which was accompanied by a marked inhibition of hepatic oxygen uptake (80% inhibition by 10 μM OXZ), various anoxic manifestations, and interference in substance exchange between the hepatocytes and perfusate [13]. The mechanism by which OXZ increases PP is not clear. The constriction of portal vein branches may be involved, since OXZ contracted extrahepatic portal vein preparations, although at concentrations higher than those observed in isolated livers [13]. Another possibility is that OXZ could narrow the hepatic sinusoids to decrease the rate of substrate exchange and oxygen extraction. Since the liver perfusion experiment was conducted in a constant flow system, the reduction in the diameters of the vein and/or the sinusoids may result in either even perfusion with a rapid flow, or uneven perfusion

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† *Abbreviations:* PP, portal perfusion pressure; ET, endothelins; TXA_2, thromboxane A_2; ANG II, angiotensin II; PAF, platelet-activating factor; OXZ, oxethazaine; KHB, Krebs-Henseleit bicarbonate buffer; FMS, fluorescence microsphere; RBC, red blood cells; MTT, mean transit time; V_{RBC}, vascular volume for RBC; and EVV_{ALB}, extravascular volume for albumin.

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by bypass formation, or both. This point needs to be examined to clarify the causal relationship between increased PP and subsequent metabolic changes, and also to characterize the action of OXZ.

For these purposes, we applied the indicator dilution method together with fluorescence microscopic observation of acridine orange-stained or FMS-trapped livers. Some experiments were done with known vasoconstrictors for comparison. The results indicated that OXZ and ET-1 caused uneven perfusion in the liver by disturbing peripheral perfusion and by forming bypasses through the branches of the proximal portal vein toward the hepatic veins.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained commercially: OXZ and azoalbumin (Sigma Chemical Co., St. Louis, MO, U.S.A.); U-46619 (Cayman Chemical Co., Ann Arbor, MI, U.S.A.); ET-1, human (Peptide Inst. Inc., Minoh, Osaka, Japan); ANG II (Bachem Inc., Torrance, CA, U.S.A.); 1-phenylephrine hydrochloride and bovine serum albumin (Wako Pure Chemical Ind., Ltd., Osaka, Japan); acridine orange (Chroma-Gesellschaft, Köngen, The Netherlands); and FluoSpheres sulfate, 1 μm, Yellow-Green and FluoSpheres polystyrene, 15 μm, Yellow-Green (Molecular Probes, Eugene, OR, U.S.A.). OXZ was dissolved in an equimolar HCl solution at a concentration of 10 mM, pH 3.5.

Animals

Male, SPF-grade Sprague-Dawley rats were purchased from Japan SLC, Hamamatsu, and housed in an air-conditioned animal room (temperature, 23 ± 1°, humidity 50–60%, and supplied with clean air) with food and water given *ad lib*. Nourished animals of 200–220 g were used throughout the experiment.

Liver Perfusion System

The livers were isolated under pentobarbital anesthesia and perfused as described previously [13]. To minimize possible uneven perfusion among liver lobes during disturbed circulation, the median and left lobes were perfused, while the other smaller lobes were tied and cut off. The portal vein and inferior vena cava were cannulated close to the liver, with the hepatic artery and branches of the portal vein being ligated and the bile duct being cut. The liver was suspended in a small vessel containing perfusion medium and perfused in a non-recirculating, constant flow (15 mL/min) system, using a rotary pump (Minipuls 2, Gilson Medical Electronics, Middleton, WI, U.S.A.). KHB (118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, and 5.6 mM glucose, saturated with 95% O_2–5% CO_2 at 37°, pH 7.4) was used as the perfusion medium. Experiments were started 30 min after portal cannulation. PP was measured using an air-trap tube (inner diameter, 3 mm; height, 50 cm) placed between the roller pump and the drug injection device. PP was initially equilibrated by setting the point of the outlet cannula to the level where the hepatic vein opens (hepatic venous pressure defined as zero). In the indicator dilution method, the effluent cannula from the liver was connected to a small hand-made flow cell through a thin teflon tube (inner diameter, 1 mm), and the outlet from the flow cell was connected to another tube (inner diameter, 2.5 mm). The final exit of the perfusate was positioned at a hepatic venous pressure zero, unless otherwise noted. The flow cell was attached to a spectrophotometer (UV-200, Shimadzu Seisakusho Ltd., Kyoto, Japan).

Indicator Dilution Method

The original theory of Meier and Zierler [14] was applied, and the multiple indicator dilution methods of Goresky [15] were modified for non-radiolabeled RBC and azoalbumin. In this experiment, the perfusate was co-infused with 2% bovine serum albumin, without which the recovery of the indicators was not satisfactory. A bolus of 25 μL of washed rat RBC (10%, v/v, suspended in the KHB containing 2% albumin) or azoalbumin (25 mg/mL KHB) was injected instantaneously into the perfusate at the portal inlet, and the optical absorbance was recorded at 650 or 370 nm, respectively. We measured the turbidity at 650 nm for RBC to avoid the possible interference from hemoglobin resulting from RBC lysis. For one liver, each indicator was injected alternately 2–3 times before and after the addition of a testing agent, and the mean values were used; in the latter case, the indicator was given after the increase in PP reached a plateau level. The absorbance was read from the chart at appropriate time intervals and converted to the concentration fraction per milliliter. The non-linear standard curve for RBC was computer-fitted, and the outflow absorbance for RBC was converted to RBC concentrations (%). MTTs for RBC and azoalbumin were computed [14] and designated as t_{RBC} and t_{ALB}, respectively, which had been corrected for the MTT for the cannula and connecting tube. The hepatic vascular and extravascular albumin spaces were calculated as V_{RBC} = flow rate (mL/sec/g liver) · t_{RBC} and EVV_{ALB} = flow rate (mL/sec/g liver) · (t_{ALB} − t_{RBC}), respectively.

Acridine Orange Staining and FMS Trapping

Acridine orange (2 μg/mL) was infused into the liver for 5 min and washed out for another 5 min. The effluent perfusate was collected during this time, and recovery was determined. The liver was soon fixed in a cold neutralized 10% formalin solution and kept for at least 24 hr. Three thick pieces were cut out from both the median and left lobes (Fig. 1, top); 50-μm slices were prepared in 0.1 M phosphate buffer (pH 7.4) containing 10% glycerol with a microslicer (DTK-1000, Dosaka EM Co., Ltd., Kyoto, Japan), mounted on slides, and photographed under a

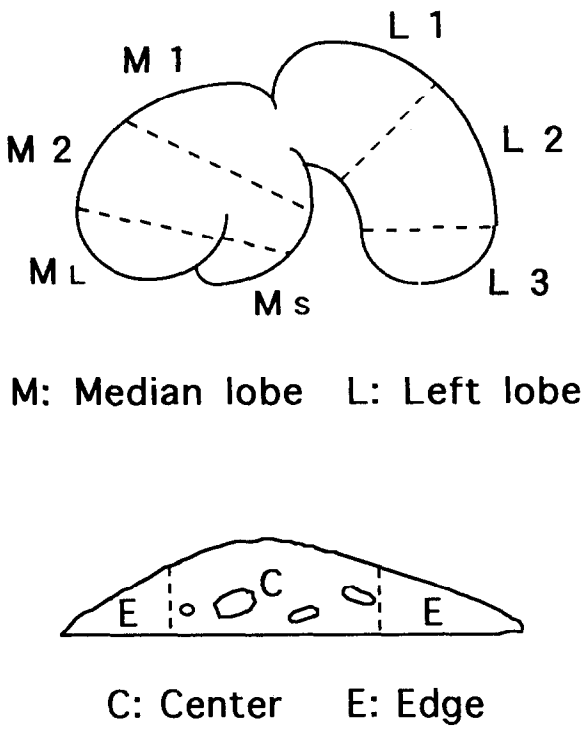

FIG. 1. Portions of the liver used for microscopic observations.

fluorescence microscope (BH2-RFCK, blue excitation, Olympus Optical Co. Ltd., Tokyo, Japan). Various parts of the slices were examined as indicated in Fig. 1 (bottom).

For FMS trapping, a bolus of 1-μm FMSs (7.5×10^7 beads in 25 μL) was injected instantaneously and the effluent was collected for 5 min to estimate the recovery. The liver was fixed, sliced, and observed under the microscope as described above. For larger beads, 15-μm FMSs (2×10^5 beads in 0.1 mL) were injected in 5 sec, and after 2 min the liver was processed in a similar fashion except that 100-μm slices were cut. In this case, no beads were recovered in the effluent perfusate.

Statistics

Data are presented as means ± SEM. Paired samples were analyzed by the paired *t*-test and unpaired samples by the *t*-test after analysis of variance by the F-test. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of OXZ on some Microcirculatory Parameters as Determined by the Indicator Dilution Method

In the control experiment (Fig. 2A, open symbols, solid lines), the outflow of azoalbumin was delayed, and its peak concentration was low compared with RBC. This is due to the distribution of azoalbumin into the space of Disse in addition to the blood vessels. Conversion of the outflow curves to a semilogarithmic scale showed somewhat biphasic decreasing patterns with both indicators (Fig. 2B). This may be due partly to the cannula and tubing space, with which similar biphasic patterns were observed (open symbols, dotted lines). Under the infusion of 20 μM OXZ (Fig. 2A and B, solid symbols and lines), RBC and azoalbumin outflows were accelerated markedly with a similar peak concentration, and their curves almost overlapped. The recovery of each indicator was above 95% without OXZ, although it tended to increase slightly but significantly under elevated PP (Table 1, upper columns).

As summarized in Table 1 (top), infusion of OXZ increased PP to three times the control level and significantly shortened both t_{RBC} and t_{ALB}; OXZ/control: 2.6/4.8 and 3.2/8.5 sec, respectively. The decrease in EVV_{ALB} (18% of the controls) was much greater than that in V_{RBC} (55%).

Since MTT and tissue spaces are expected to vary with

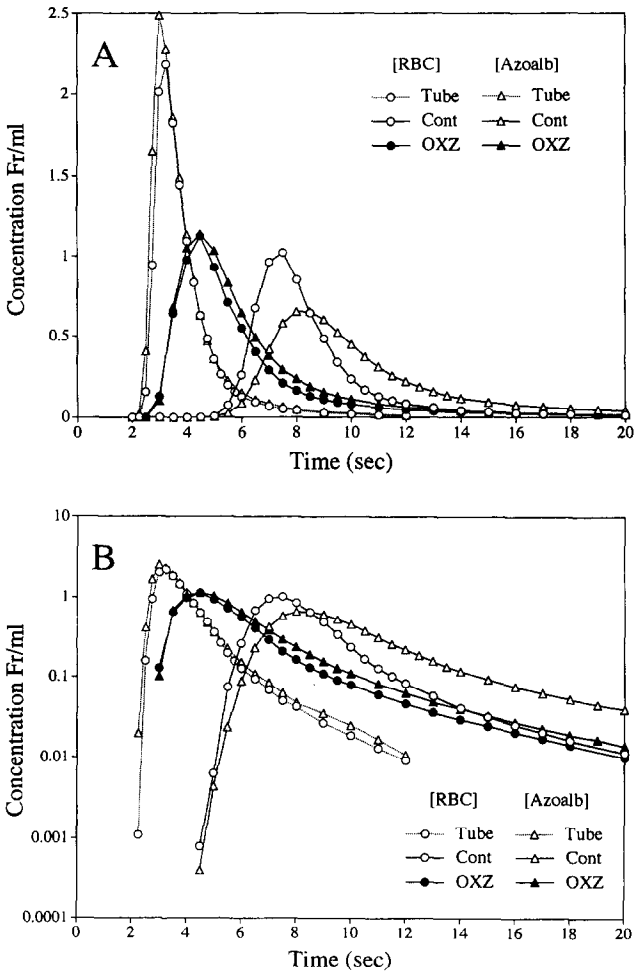

FIG. 2. Effects of OXZ on the outflow curves of RBC and azoalbumin in the isolated perfused rat liver, shown in linear (A) and semilogarithmic (B) plots. Indicators were injected before OXZ infusion (controls) and then when PP was elevated by 20 mM OXZ. Dotted lines indicate fast outflow patterns from the cannula and the connecting tube space. Representative data from 10 experiments (Table 1, top) are shown. Details are described in Materials and Methods.

TABLE 1. Effect of OXZ on the portal pressure and some microcirculatory parameters obtained by the indicator dilution method in the perfused rat liver under different hepatic vein pressures

Perfusion conditions	OXZ (20 μM)	PP (cm H_2O)	MTT (sec)		Recovery (%)		V_{RBC} (mL) (%)	EVV_{ALB} (mL) (%)
			t_{RBC}	t_{ALB}	RBC	ALB		
Regular HVP (N = 10)	−	9.2 ± 0.1	4.77 ± 0.38	8.45 ± 0.49	98.2 ± 1.0	96.2 ± 1.1	0.21 ± 0.02 (100)	0.17 ± 0.01 (100)
	+	29.9 ± 1.5*	2.57 ± 0.16*	3.19 ± 0.15*	105.4 ± 1.8†	106.7 ± 1.5*	0.11 ± 0.01* (55.0)	0.03 ± 0.01* (18.2)
High HVP (N = 7)	−	12.8 ± 0.6	9.40 ± 0.21	18.10 ± 0.35	93.3 ± 1.5	91.2 ± 1.8	0.39 ± 0.01 (100)	0.37 ± 0.01 (100)
	+	24.0 ± 1.9*	5.12 ± 0.28*	6.51 ± 0.38*	100.8 ± 1.1‡	98.8 ± 2.0‡	0.21 ± 0.01* (54.5)	0.07 ± 0.02* (17.7)
Low HVP (N = 6)	−	9.3 ± 0.1	10.78 ± 1.53	4.73 ± 0.17	72.0 ± 2.0§	94.2 ± 1.2		
	+	29.3 ± 1.2*	2.70 ± 0.18†	2.52 ± 0.24*	106.0 ± 2.3*	108.6 ± 1.1*	0.13 ± 0.01	0 ± 0.01

Abbreviations: HVP, hepatic venous pressure; PP, portal pressure; MTT, mean transit time; t_{RBC} and t_{ALB}, MTT for RBC and albumin, respectively; and V_{RBC} and EVV_{ALB}, vascular and extravascular volume for RBC and albumin, respectively.

The liver was perfused in a non-recirculating manner at a flow rate of 15 mL/min. In the regular HVP, the tip of the exit tube was positioned at HVP zero. The tip position was raised about 3 cm in the high HVP group and lowered 4 cm in the low HVP group. The t_{RBC} and t_{ALB} values have been corrected for cannula and tube space. V_{RBC} and EVV_{ALB} were calculated using the MTT values. Values represent the means ± SEM.

*, †, ‡ Significantly different from the minus OXZ group at: $*P < 0.001$, $\dagger P < 0.01$, and $\ddagger P < 0.05$.

§ Significantly lower than the other control groups, $P < 0.001$.

portal and hepatic vein pressure changes, the effects of OXZ were examined under different portal and venous pressure. At high PP (+3 cm H_2O), which was produced by increasing the hepatic venous pressure by raising the exit level, the MTT and tissue spaces increased to about twice the control values, and the effect of OXZ was still evident (Table 1, middle). Conversely, when the hepatic venous pressure was decreased by lowering the exit level by 4 cm, the PP in the control liver was not affected much, while t_{RBC} was about twice as long with a significant decrease in RBC recovery, but t_{ALB} was shortened by half. This may result from mechanical narrowing, due to the negative pressure of the sinusoids, which impedes RBC flow. Even under these conditions, OXZ was still effective. These observations confirm that the action of OXZ is substantial and independent of the perfusing pressure.

Experiments with Some Known Vasoconstrictors

The vasoconstrictors tested decreased the MTTs of RBC and azoalbumin and their tissue volumes to variable degrees (Table 2). As compared with OXZ, phenylephrine and ANG II had lesser effects on MTTs and tissue spaces, especially with t_{ALB} and EVV_{ALB}; decreases in the EVV_{ALB} values for phenylephrine and ANG II (89 and 62% of the controls, respectively) were much smaller than that for OXZ (18%). UTP caused somewhat greater decreases in t_{ALB} and EEV_{ALB} (33%). With these three agents, however, the maximum PP response was less than that of OXZ. In this respect, U-46619 and ET-1 increased PP to a level comparable to OXZ. Under such conditions, however, U-46619 had less effect on the MTT and tissue spaces (V_{RBC} and EVV_{ALB}; 74 and 42% of the controls, respectively) than OXZ, whereas ET-1, like OXZ, markedly decreased V_{RBC} and EVV_{ALB} to 43 and 11% of the controls, respectively.

The results of the indicator dilution method alone imply that OXZ and the vasoconstrictors tested, except phenylephrine, decreased not only hepatic vascular space but also the extravascular Disse space. However, this method provides information on hepatic circulation as a whole but cannot exclude a localized circulatory perturbation. We therefore attempted microscopic examinations. In the following experiments, the concentration of OXZ was lowered to 10 μM in the absence of albumin in the perfusate.

Effects of OXZ on Acridine Orange Staining

The fluorescent dye acridine orange was infused in the absence and presence of OXZ, U-46619 (0.5 μM), and ET-1 (5 nM) for comparison, all of which increased the PP to a similar degree, i.e. 34.8 ± 1.5, 34.5 ± 1.5, and 35.9 ± 0.3, respectively, vs 9.0 ± 0.2 cm H_2O in the controls, N = 3–4, with slightly higher responses being observed when there was no albumin in the perfusate. In the control livers, the acridine orange injected was not eluted out, whereas under the conditions where PP was elevated, the dye was recovered in the effluent to the following values, in increasing order: U-46619 (11.9 ± 1.8%) < ET-1 (20.4 ± 3.4%) < OXZ (30.9 ± 2.5%).

In the fluorescence microscopy studies, the control livers infused with acridine orange revealed that all periportal zones were evenly stained throughout the median and left lobes, but the midzonal and pericentral areas were not stained (Figs. 3A, 4A and 4F), which is in accord with observations *in vivo* [16]. However, with OXZ infusion,

TABLE 2. Effects of some vasoconstrictors on portal pressure and microcirculatory parameters obtained by the indicator dilution method in the perfused rat liver

Perfusion conditions	Drugs	PP (cm H_2O)	MTT (sec)		Recovery (%)		V_{RBC} (mL) (%)	EVV_{ALB} (mL) (%)
			t_{RBC}	t_{ALB}	RBC	ALB		
Phenylephrine, 10 μM	−	9.2 ± 0.1	5.58 ± 0.48	9.19 ± 0.69	98.4 ± 1.6	95.1 ± 1.6	0.27 ± 0.03 (100)	0.19 ± 0.03 (100)
	+	21.2 ± 1.6*	3.68 ± 0.32*	6.91 ± 0.79*	93.7 ± 1.8†	100.5 ± 0.6†	0.18 ± 0.02* (66.5)	0.17 ± 0.03‡ (88.5)
ANG II, 50 nM	−	9.1 ± 0.1	4.80 ± 0.19	9.15 ± 0.54	95.3 ± 1.5	97.1 ± 0.5	0.25 ± 0.02 (100)	0.23 ± 0.02 (100)
	+	22.4 ± 1.2‡	3.26 ± 0.12‡	5.87 ± 0.17*	88.5 ± 2.7†	99.2 ± 0.9	0.17 ± 0.01* (68.1)	0.14 ± 0.01* (62.1)
UTP, 100 μM	−	9.0 ± 0	4.91 ± 0.27	8.31 ± 0.72	97.1 ± 2.6	93.8 ± 1.9	0.23 ± 0.01 (100)	0.16 ± 0.02 (100)
	+	25.8 ± 1.5‡	3.41 ± 0.38‡	4.37 ± 0.59*	98.4 ± 2.1	102.2 ± 2.8*	0.16 ± 0.02‡ (68.5)	0.05 ± 0.01* (33.3)
U-46619, 500 nM	−	10.1 ± 0.9	5.79 ± 0.12	10.70 ± 0.31	96.6 ± 1.2	96.6 ± 1.2	0.26 ± 0.01 (100)	0.23 ± 0.02 (100)
	+	28.9 ± 1.2‡	4.29 ± 0.32†	6.33 ± 0.21‡	97.4 ± 1.0	98.3 ± 1.0	0.20 ± 0.02† (74.3)	0.09 ± 0.01* (42.4)
ET-1, 5 nM	−	9.1 ± 0	5.51 ± 0.32	9.87 ± 0.34	100.7 ± 2.8	97.8 ± 3.4	0.23 ± 0.01 (100)	0.20 ± 0.01 (100)
	+	29.2 ± 1.6‡	2.35 ± 0.13‡	2.54 ± 0.15‡	109.1 ± 2.2†	111.4 ± 5.3	0.10 ± 0.01‡ (43.4)	0.02 ± 0.01‡ (11.1)

Abbreviations: ANG II, angiotensin II; ET-1, endothelin 1; for others, see Table 1. Values represent the means ± SEM, N = 5.
*, †, ‡ Significantly different from the controls at: *$P < 0.01$, †$P < 0.05$, and ‡$P < 0.001$.

uneven fluorescent staining was evident. Extensive staining was mostly observed to be localized to the central portion of the lobes, whereas the periphery of the liver was nearly unstained. About 70–80% of the liver section appeared unstained (Fig. 3B). Strong staining often was observed around large portal vein branches and spread toward large hepatic veins, and the periportal areas of the smaller portal vein branches at the periphery were either weakly stained or not stained at all (Fig. 4, B and F). The large portal tracts are known to have many small side branches directly connecting to the sinusoids [17, 18], and the dye may flow into the sinusoids via such vascular systems. The staining changes induced by OXZ recovered 30 min after the 30-min OXZ infusion period (Fig. 4C).

ET-1 also altered the staining pattern as observed with OXZ (Fig. 4D). However, U-46619 caused fewer changes, and some portal areas in the liver periphery were still stained (Fig. 4E).

Effects of OXZ on FMS Trapping

Two FMS sizes were used. In the control liver infused with 1-μm FMSs, an unexpectedly high recovery (46.4 ± 0.49%, N = 3) was observed in the effluent perfusate, and the remaining beads were trapped evenly throughout the liver section (Fig. 5A). OXZ increased the recovery (72.6 ± 3.9%, N = 3), and the FMSs were largely trapped in the central portion of the liver, with minimal amounts trapped in the periphery (Fig. 5B).

With the 15-μm FMSs, no beads were recovered in the effluent regardless of the OXZ treatment. In the control livers, FMSs were scattered mainly in the periportal zones, and some were found in the midzonal areas throughout the whole liver (Fig. 5C). However, in the OXZ-treated livers, FMSs were localized and clustered along the portal canals or in the periportal zones, especially in the central portions of the liver, and almost no beads appeared in the periphery (Fig. 5D). These observations with FMSs are consistent with those with acridine orange, confirming that OXZ markedly disturbs peripheral circulation in the liver.

DISCUSSION

First, the methodology used in this study, a combination of the indicator dilution method and microscopic observation of acridine orange staining and microsphere trapping, may be suitable for evaluating perfusion disturbance in the isolated perfused liver. Compared with the multiple indicator (radiolabeled) dilution method described by Goresky [15], the on-line absorbance recording of the outflow of RBC and azoalbumin is only applicable to the artificially perfused liver, but this is easily done. However, perfusing conditions such as portal and hepatic venous pressure affect the outflow profiles, and the addition of albumin to the

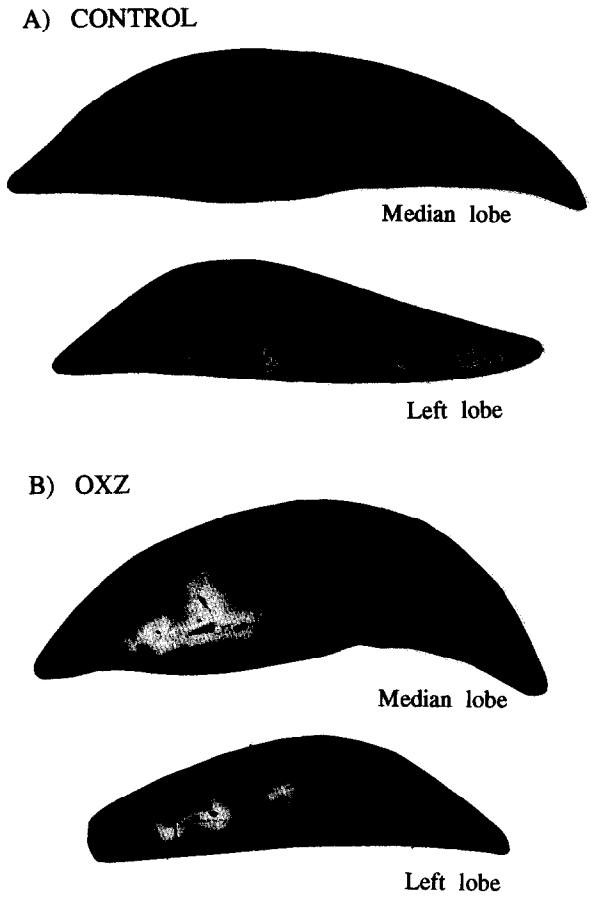

FIG. 3. Whole fluorescent microscopic pictures of cross sections of the liver stained with acridine orange. (A) The control perfused liver. (B) Under conditions where the PP was elevated by 10 μM OXZ. Magnification: ×2.1.

perfusate is necessary for satisfactory recovery of the indicators.

However, the application of indicator dilution methods rests on several assumptions; for example, all the materials injected into the liver must traverse the liver, i.e. there must be no shunt [1]. Shunt formation causes underestimation of tissue spaces. Since shunts were found to be formed by OXZ, ET-1, or U-46619 through the microscopic observations in the present study, the calculated tissue spaces under the effects of these agents are erroneous, even though the data from indicator dilution studies prompted us to examine intrahepatic flow disturbance. By application of the indicator dilution method, Lapointe and Olson [9] reported a decrease in hepatic sucrose, urea, and RBC spaces during PAF infusion in the perfused rat liver, in which they did not confirm even perfusion of the liver in the presence of the agent although the possibility of uneven perfusion has been discussed. In a similar experiment, Reichen [19] also reported a decrease of hepatic spaces by norepinephrine at maximally effective concentrations. From our results, under vasoconstriction, confirmation of the even perfusion of the liver is obviously of primary importance. In addition, with a constant flow system, the increased driving force due to vasoconstriction may bias MTTs to higher values by counteracting a vasoconstrictive effect. Thus, the indicator dilution study under vasoconstriction should be interpreted carefully.

Acridine orange has been used *in vivo* to examine liver microcirculation by epi-fluorescence microscopy [16]. However, the information from this method is confined to the surface areas and cannot determine the overall flow change inside the liver mass, which may secondarily affect the surface flow pattern. We also tested this method but could deduce no clear conclusion. Alternatively, microscopic observation of fixed liver sections is adequate for studying intrahepatic circulatory disturbances. This method may also be applied to *in vivo* experiments. The microsphere trapping method has been widely used to examine bypass formation in the cirrhotic liver [20–23]. The diameters of rat sinusoids reportedly range from 6 to 14 μm with a smaller diameter in zone 1 [24], while the diameters of endothelial fenestrae in the sinusoids range from 0.1 to 0.2 μm [24]. The trapping of a considerable amount of 1-μm FMSs in this study was unexpected, and may be due to a surface charge interaction between FMS and the sinusoidal inner surfaces and partly to phagocytosis by Kupffer cells.

Second, when the PP was increased by OXZ, the liver was unevenly perfused with little or no perfusion in the peripheral portion, and about 70–80% of the liver mass was not well perfused. This may be largely concerned with the decreases in the MTT and tissue spaces observed in the indicator dilution experiment. We further confirmed the disturbed peripheral perfusion by epi-fluorescence microscopic observation of the liver surface under 0.5-μm FMSs infusion, in which the surface flow of the FMSs stopped soon after the infusion of OXZ. This uneven perfusion flow may be the primary reason for decreased oxygen uptake and the subsequent metabolic effects as well as the diminished substrate exchanges produced by OXZ in the perfused liver. OXZ at concentrations used in this study did not cause respiratory inhibition in isolated mitochondria and hepatocytes [13].

OXZ contracted an extrahepatic portal vein preparation, though at concentrations 10 times higher than those that increased PP [13]. Smaller portal vein branches may be more sensitive. Thus, the biased flow around the large portal veins in the center of the liver may be explained by (1) collective constriction of the peripheral small portal branches, (2) a pressure increase in the large portal veins, and then (3) bypass formation through shorter marginal branches of the portal trunks to the hepatic veins. Constriction of the sphincters, which are proposed to exist in terminal portal venules and regulate hepatic microcirculation [25, 26], could also be involved. In this connection, the intrahepatic shunt in the cirrhotic liver could be formed in a similar manner with the development of fibrosis.

Although it is still controversial [27], sinusoids are also thought to constrict, based on the fact that cultured

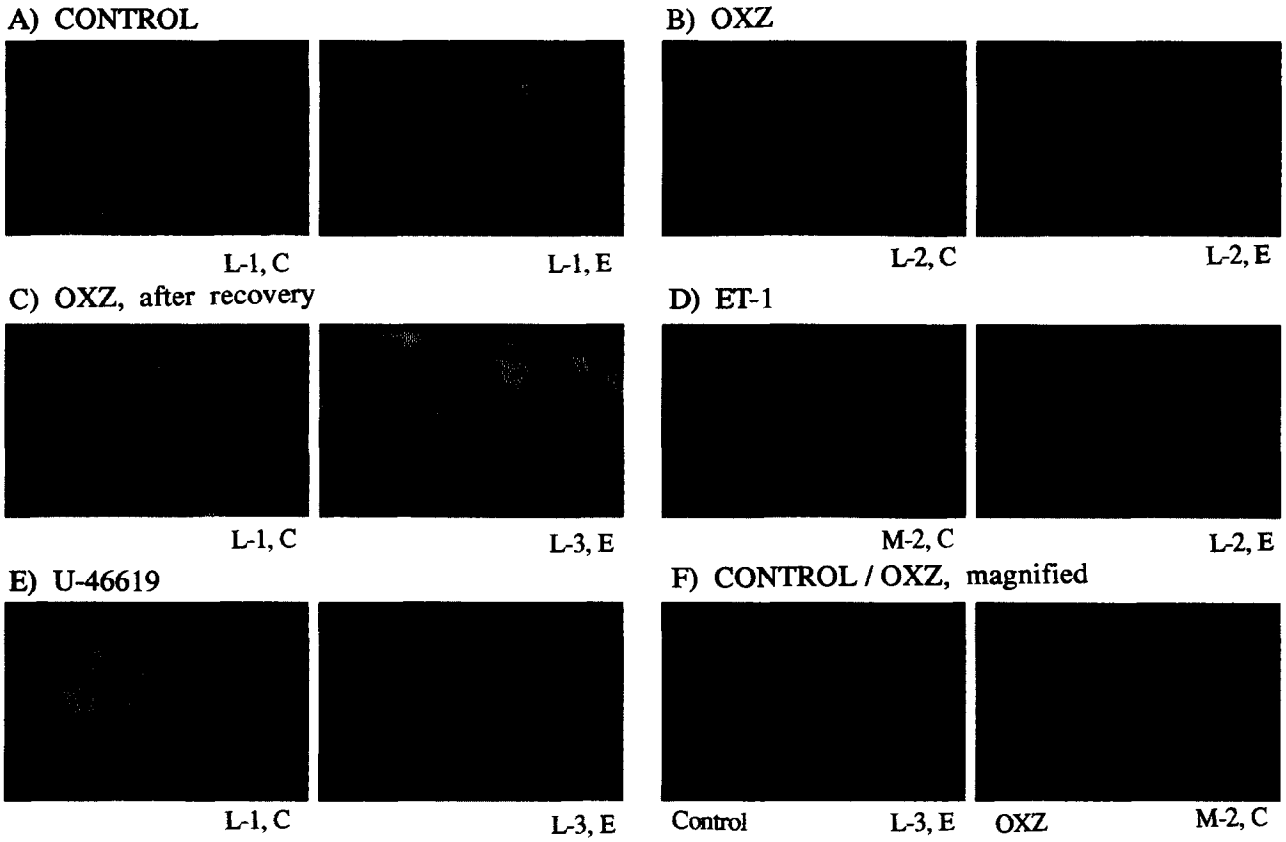

FIG. 4. Pictures of the liver sections stained with acridine orange. (A) Control livers (N = 3). (B) PP increased by 10 μM OXZ (N = 4). (C) After recovery from the actions of OXZ; 30 min after 30-min of infusion of 10 μM OXZ (N = 2). (D) PP increased by 5 nM ET-1 (N = 3) (E) PP increased by 500 nM U-46619 (N = 3). (F) Magnification in A and B. Representative pictures at various portions were selected. Abbreviations of the liver portions are shown in Fig. 1. Magnifications: A–E, ×9.4; F, ×23.5.

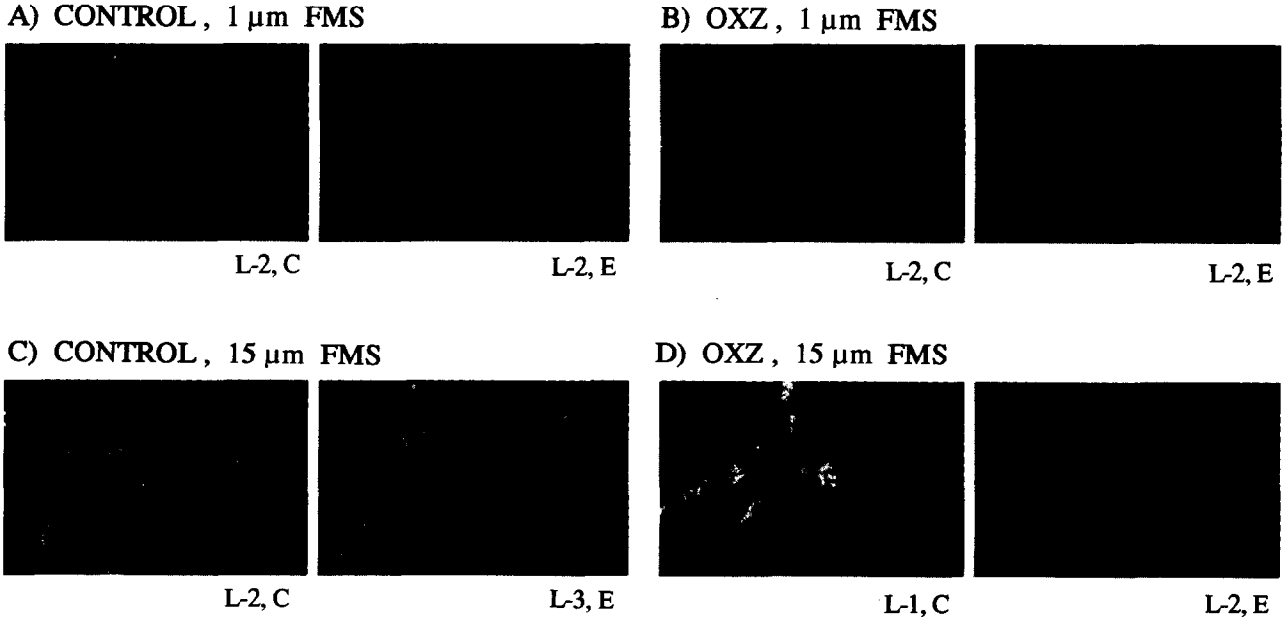

FIG. 5. Effect of OXZ on fluorescent microsphere (FMS) trapping in the perfused rat livers. (A) 1-μm FMS, control liver; (B) 1-μm FMS, OXZ-treated liver; (C) 15-μm FMS, control liver; and (D) 15-μm FMS, OXZ-treated liver. Representative results from 2 to 3 livers are shown. Abbreviations of the liver portions are shown in Fig. 1. Magnification: ×9.4.

sinusoidal Ito cells can be transformed to contractile myoblasts, and contraction is stimulated by various substances including ET-1 [28–31]. Sinusoidal endothelial cells also contain contractile elements, and their fenestrae are reported to change in size [32–34]. By *in vivo* epi-fluorescence microscopy, ET-1 is proposed to act at both extrasinusoidal and sinusoidal sites [35]. However, it is still uncertain whether OXZ constricts the sinusoids. The fact that, under negative hepatic venous pressure which probably narrows the sinusoids, the PP response caused by OXZ was comparable to that under regular conditions may favor the vascular action of OXZ.

Third, as described in the introduction, the causal relationships between the hemodynamic and metabolic changes produced by various vasoconstrictors have not been well defined. Uneven perfusion of the liver may be one of the possible causes, as shown by OXZ, ET-1, and U-46619. In the case of U-46619, peripheral perfusion was relatively well preserved, despite PP increases comparable to those of OXZ and ET-1. This suggests that the disturbance of hepatic microcirculation by U-46619 is mechanistically different than that caused by OXZ or ET-1. From this point also, microscopic examination of the fixed liver may be useful for characterizing microcirculatory changes.

Lastly, the action of OXZ in the perfused rat liver appears to be unique, since other local anesthetics such as procaine, dibucaine, lidocaine, and tetracaine have no such actions [13]. In addition, (1) unlike norepinephrine, OXZ was not antagonized by prazosine or by sodium nitroprusside, (2) under retrograde perfusion, the PP increase induced by OXZ was abolished, whereas norepinephrine, UTP, ANG II, and ET-1 were still effective, and (3) the TXA_2 antagonist SQ-29548 did not antagonize OXZ [13]. The PAF antagonist 1-*O*-hexadecyl-2-*O*-acetyl-*sn*-glycero-3-phospho(*N*,*N*,*N*-trimethyl)hexanolamine and ET-1 antiserum also did not block the actions of OXZ (our unpublished observations). Further mechanisms of the actions of OXZ are now being studied.

It is unlikely that the actions of OXZ observed in the isolated liver are clinically operative at therapeutic dosages, e.g. 15–40 mg/day, divided into 3–4 administrations, p.o. Toxicological studies with animals also showed that oral administration of the drug has a considerable margin of safety; however, toxicity following intravenous injection is high, with accompanying impairment of myocardial contractility and conduction [36]. Anyway, it is interesting that in the doses employed, OXZ produced portacaval shunting in the perfused liver.

In conclusion, the increase in PP and subsequent metabolic effects exerted by OXZ in the perfused liver may result primarily from the contraction of the peripheral vascular system and bypass formation. The methodology used in this present study may help elucidate hepatic perfusion disturbances caused by various vasoconstrictors.

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