Anthracycline do not inhibit the enzymes of the reduction system (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase, glutathione peroxidase, catalase, superoxide dismutase) of the erythrocytes[3]. Peroxidative damage to erythrocyte membrane proteins and lipids may have a role in the inhibition of ATPase. Indeed, ATPase is known to be lipid dependent. However, another lipid dependent membrane enzyme, acetylcholinesterase is not inhibited by aclacinomycin. Ascorbate is essential to a variety of biological oxidation systems. The most prominent chemical property is its ready oxidation to dehydroascorbic acid. Massive dose of ascorbate may prevent the membrane protein and lipid from peroxidative damage, however the definite generation of reactive oxygen species in intact erythrocytes has not been demonstrated. The erythrocyte model we have described might be useful in the detection and screening of additional compounds capable of producing such radicals. Although our in vitro data show that the effects of aclacinomycin on erythrocytes are dependent on concentration and time of incubation, the clinical use of aclacinomycin would not be expected to affect erythrocytes except perhaps transiently after i.v. administration. However, the possibility that administration at high dosage schedules may cause sufficient oxidative injury to erythrocytes to result in hemolysis should be considered. Pretreatment with massive ascorbate before administration of aclacinomycin may produce semiquinone effectively and reduce lipid peroxidation in the erythrocyte. Ascorbate may ameliorate reverse effects of aclacinomycin without impairing tumor response.

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Stimulation of cyclo-oxygenase by lidocaine, a pro-lipoperoxidant

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Summary. Lidocaine, which was recently demonstrated to be a good pro-lipoperoxidant, was tested on in vitro PGs biosynthesis, and on arachidonate-induced arterial hypotension, in the rabbit. In the in vitro experiments, lidocaine alone was a poor stimulant of cyclo-oxygenase, but it enhanced significantly the cyclo-oxygenase activation of uric acid. In the rabbit, lidocaine lowered the i.v. arachidonate dose necessary to obtain a significant drop of blood pressure.

In 2 preliminary communications, we demonstrated that lidocaine has a pro-lipoperoxidant effect in that it accelerates the arachidonic acid autoxidation and the decomposition of arachidonic lipoperoxides into malonaldehyde[1,2].

It is well known that lipoperoxides are necessary to trigger and to maintain the cyclo-oxygenase activity[3]. Therefore, it would be of interest to know the effect of lidocaine on that enzyme activity in 'in vitro' and 'in vivo' conditions. To answer those questions, we have performed 2 kinds of experiment. First, we have studied the influence of lidocaine on prostaglandin biosynthesis by microsomes of bull seminal vesicles. In order to avoid the auto-inactivation of cyclo-oxygenase by excited oxygen species during the production of peroxides, uric acid, a potent free radical scavenger[5], was added to the reaction medium. In a second kind of experiment, we observed the arachidonate-induced hypotension in rabbits treated or not treated with lidocaine.

Material and methods. In vitro PGs biosynthesis. Microsomes of bull seminal vesicles were prepared following a procedure published elsewhere[6]. 14-C arachidonate was incubated in the presence of glutathione (10^{-4} M), in a phosphate buffer (6.10^{-2} M) at pH 7.8. After extraction with diethylether, the prostanoids were separated on TLC plates, and the labeled spots were visualized by autoradiography and automatically eluted by Eluchrom devices. Radioactivity counting was performed using a Nuclear Chicago beta scintillation counter. Uric acid was carefully dissolved in a phosphate buffer (pH 7.8), heated in a water bath at 40 °C and then added to the medium at a final concentration of 10^{-3} M. Lidocaine (Xylocaine®) was also used, at the same final concentration.

Arachidonate-induced hypotension in a rabbit model. This model has been described elsewhere[6,7]. It consists of rabbits anesthetized with nembutal (40 mg/kg) and heparinized i.v. (3 mg/kg) 1 h before the assays. They were intubated and artificially ventilated by a Servo-Ventilator Siemens Elema 900 B respirator, with an N_2/O_2 mixture. A 22% FiO_2 was maintained, monitored by a polarographic gauge. Arterial blood pressure, venous pressure and electrocardiogram were continuously registered by a Sanborn 7700 device.

In heparinized rabbits, after 1 h, the AA_{50} (see fig.2) falls from 400–500 µg/kg to ± 150 µg/kg[6,7], and the animals can be used for in vivo arachidonate conversion studies.

To compare the effects of different agents on the AA_{50}, we use a sensitization coefficient. This is the number by which the previous AA_{50} must be divided to obtain the new AA_{50}, observed after the administration of the agent. The larger the sensitization coefficient, the more the agent stimulates the in vivo arachidonic acid conversion.

The results obtained with this animal model may be falsified if the animal is affected by an inflammatory process, enhancing the plasmatic anti-inflammatory proteins. In such case, arachidonate conversion into prostanoids is strongly impaired. Before using a rabbit, we check the plasma haptoglobin level, as described earlier. Haptoglobin is not active by itself, but is a good index of the presence of cyclooxygenase inhibitors in the plasma. We consider that rabbits are unsuitable for this animal model when their haptoglobin level exceeds 30 mg% HbBC[9].

Results. In vitro biosynthesis of PGs. As shown in figure 1, lidocaine used alone is a poor stimulator of cyclo-oxygenase activity. At 10^{-3} M, its enhancing effect is represented by a coefficient of 1.13. Uric acid has a more important effect on PGs biosynthesis than lidocaine. At 10^{-3} M, it enhances the rate of PG synthesis by a coefficient of 1.8. But lidocaine strongly potentiates the stimulant action of uric acid. The combination of the 2 drugs has a synergic effect on PGs biosynthesis rate, which is increased by a coefficient of 2.5.

Arachidonate-induced hypotension in the rabbit. 1 h after heparinization of the rabbit, the AA_{50} (i.v. arachidonate dose required to produce a 50% drop of the mean arterial pressure) decreases from 400–600 µg/kg to 130–170 µg/kg. This new AA_{50} then remains unchanged for at least 5 h. I.v. lidocaine, administered in a single dose, elicits a drop of the AA_{50} to about 10–20 µg/kg.

As shown in the table, the sensitization coefficient is dose-dependent, vs lidocaine, only below 25 mg/kg. Up to this value, this coefficient fluctuates around 4.5; it ceases to be dose-dependent. But the stimulant effect of a single dose of lidocaine on the arachidonic hypotension is maintained at its maximum during a limited time, proportional to the dose of lidocaine. On the figure 2, an example is given, showing the efficiency of the action of lidocaine on the decrease of the AA_{50} in the rabbit.

Discussion. Hemler et al.[3] have underlined in the past few years the key role played by peroxides and hydroperoxides in triggering PG biosynthesis. However, the stimulating effect is difficult to observe because OH˙, or small alkoxy radicals which are generated at the same time, inactivate cyclo-oxygenase. According to Egan et al.[4] cyclo-oxygenase activity is prolonged if free radical scavengers, such as methional, are added to the medium. This finding was confirmed by the use of more active radical scavengers than those used by Egan[5], such as uric acid.

Thus 2 opposite effects are exerted on cyclo-oxygenase; a stimulating effect due to lipoperoxides and an inhibiting effect due to small radicals. Lidocaine is a pro-lipoperoxidant agent and increases lipoperoxide production[1]. Used alone, it slightly enhances PG biosynthesis. When a small radical scavenger such as uric acid is added, the inhibiting effect on cyclo-oxygenase activity is abolished and the stimulating action of lidocaine appears more clearly.

That lidocaine induces a cyclo-oxygenase depletion, by hyperstimulation of this enzyme, was demonstrated on stomach strips[2,9]. A rapid tachyphylaxis occurs when arachidonic acid is employed together with lidocaine. But this tachyphylaxis is attenuated by uric acid, and then the stimulant property of lidocaine on arachidonate conversions appears clearly[9]. The attenuation by uric acid of this tachyphylactic phenomenon was also observed by Bourgain et al.[10], during in vivo experiments on thromboformation in the rat.

The effect of lidocaine on arachidonate-induced hypotension in the rabbit seems to be related to a better conversion of this precursor into hypotensive icosanoids, such as prostacyclin. Fletcher et al.[11] reported recently that lidocaine favors the prostacyclin pathway, in baboons and in rats[12], to the detriment of the thromboxane path. However, these

I.v.dosis of lidocaine	Number of rabbits	Sensitization coefficient	Efficiency duration
10 mg/kg	2	2.1	11 min
		2.4	9 min
25 mg/kg	3	5.1	18 min
		4.8	16 min
		4.4	21 min
100 mg/kg	2	4.1	42 min
		5.2	54 min
200 mg/kg	2	4.2	160 min
		3.9	210 min

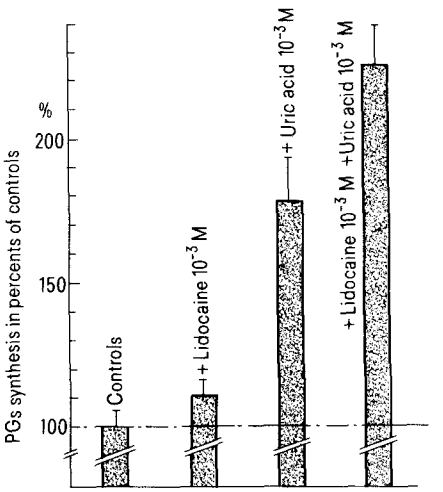

Figure 1. In vitro biosynthesis of PGs; stimulation by lidocaine. Lidocaine (at 10^{-3} M) has a moderate stimulating effect on biosynthesis of PGs by bull seminal microsomes (110%). But, used in continuation with uric acid (at 10^{-3} M), it stimulates the biosynthesis greatly (225%). The pro-lipoperoxidant activity of lidocaine and the radical scavenging activity of uric acid are responsible for these effects.

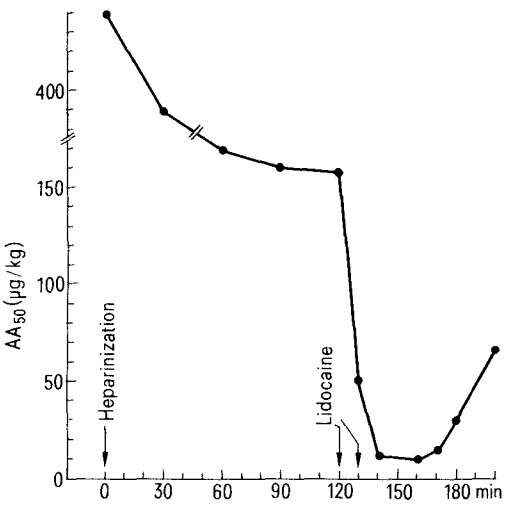

Figure 2. Sensitization of the rabbit to the vasodepressor effect of arachidonate by lidocaine. AA_{50} arachidonate i.v. dosis (in µg/kg) required to induce a 50% fall of the mean arterial pressure. Heparin (injected at time O, at 2 mg/kg) decreases the AA_{50} to about 150 µg/kg. After lidocaine injection (100 µg/kg), the AA_{50} falls to 10 µg/kg for a 40-min. period.

authors cannot give any satisfactory explanation of these phenomena. We propose here 3 mechanisms for the enhanced prostacyclin plasma concentration observed by Fletcher et al.

1. A cyclo-oxygenase stimulation, in the presence of a natural free radical scavenger, such as uric acid, resulting from the ability of lidocaine to accelerate lipoperoxidation[1]. 2. The inhibition, by lidocaine, of the 15-OH-prostaglan-deshydrogenase, described by Tai et al.[13], may contribute to enhancing the tissue and plasma prostanoid concentrations. 3. The impairment of phospholipasic activities by lidocaine[14,15] is also possible. However, it must be underlined that we employed exogenous arachidonate, so that this mechanism must be excluded in our experiments.

Conclusions. The pro-lipoperoxidant effect of lidocaine is associated with a stimulant action on cyclo-oxygenase activity, observable particularly in the presence of a radical scavenger, such as uric acid. This property is confirmed by in vitro and in vivo experiments. However, in in vivo conditions, lidocaine may enhance PGs biosynthesis by another pathway, such as phospholipase and PG 15-OH-deshydrogenase inhibition.

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Anaerobic metabolism during activity in the rainbow trout *(Salmo gairdneri)*

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Summary. Rainbow trout 27 g b.wt were trained to swim in a water tunnel at 1.1 body lengths · sec^{-1} (10 °C). Swimming speed was increased over 60 sec to either 2.3, 3.8, 5.3, 6.1 or 7.0 body lengths · sec^{-1} and fish were freeze clamped in liquid nitrogen. Other fish were sampled after a further 5 min steady swimming. Anaerobic energy production (mmoles · ATP · kg^{-1} min^{-1}) calculated from whole body lactate concentrations increased from 0.23 at 2.3 body lengths sec^{-1} to 1.6 at 7.0 body lengths sec^{-1}. Lactate concentrations decreased for periods of swimming greater than 20 min partly due to a catabolism of lactate.

The energy source for swimming is critically dependent on both the intensity and duration of effort[2]. For reptiles and amphibians whole body lactate analysis has been used to determine the importance of anaerobic metabolism during activity[3,4]. In rainbow trout, only the anaerobic contribution to initial activity may be calculated due to a significant catabolism of lactate under steady state conditions[5].

Materials and methods. Rainbow trout (*Salmo gairdneri* Richardson), 13.4 ± 0.2 cm length and 27.2 ± 1.5 g b.wt were obtained from North East Fife Fish Farm, Scotland. They were held in fresh water and fed on proprietary trout pellets. Swimming experiments were carried out in an open-top flume (150 cm long × 25 cm diameter) as described by Johnston and Moon[6]. Temperature in both holding tanks and exercise chamber was maintained at 10 ± 0.5 °C. Groups of 6–8 fish were conditioned to swimming in the chamber at 1.1 body lengths · sec^{-1} for at least 3 days prior to experiments. Following this training period, water flow was increased to speeds equivalent to 2.3, 3.8, 5.3, 6.1 or 7.0 body lengths · sec^{-1} over a period of 60 sec and half the fish were sampled. Other groups of fish were allowed to swim for a further 5 min prior to sampling. Only fish exhibiting normal swimming behavior were sampled. Fish that struggled or fell back against the restraining barrier (~ 10%) were removed from the chamber and discarded. Fish were stunned and freeze-clamped in liquid nitrogen (−159 °C) as previously described[5]. Although a finite time is required to freeze the whole carcass, both initial and final samples were treated in a similar manner and are subject to the same errors. Lactate was determined in duplicate from perchloric acid extracts prepared from the whole carcass[5]. Preliminary experiments established that lactate concentrations continued to increase during the 1st 8–20 min swimming and thereafter declined as steady state conditions were obtained.

Results. Total body lactate concentration was 7.9 ± 0.5 μmoles · g^{-1} in fish swimming at 1.1 body lengths · sec^{-1}. Figure 1 shows lactate concentrations after acceleration to either 2.3, 3.8, 5.3, 6.1 or 7.0 body lengths · sec^{-1} and following a further 5 min swimming. The rate of lactate production was much higher during acceleration than during steady swimming, varying from 3.8 mmoles kg^{-1} · min^{-1}