The results thus indicate that it is possible to modify the antitumor efficacy of FT in a particular direction by means of PFD, an inducer of the liver cytochrome P-450 system. A combination of FT with PFD potentiates the action of FT on tumors resistant to it (LLC, hepatoma H-2-73) on average by a factor of 2.5. The possibility of significantly reducing the dose of FT, which exhibits marked neurotoxicity, without any loss of antitumor efficacy is evidence that the use of such combinations in clinical practice is promising.

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SOME MOLECULAR MECHANISMS OF THE ANTIOXIDATIVE ACTION OF DALARGIN ON THE LIVER IN EXPERIMENTAL CHOLESTASIS

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Dalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), a synthetic analog of the opioid Leu-enkephalin, synthesized in the Laboratory of Peptide Synthesis, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, by Professor M. I. Titov, and which possesses antistressor activity and exerts a protective effect on organs (including the liver), has begun to be used in recent years as a protective agent in anesthesiology. In such cases dalargin has been observed not to have a direct membrane-stabilizing effect on the myocardium [4], and the protective action of dalargin (in myocardial infarction, after wounding) has been shown to be realized in opioidergic receptor processes, for it is completely abolished by simultaneous administration of the structural morphine analog naloxone, a universal opioid antagonist [1, 7].

Meanwhile, the molecular mechanisms of the protective action of dalargin on the liver have not been adequately studied. There is no information in the literature on the action of the dalargin antagonist, naloxone, on liver function.

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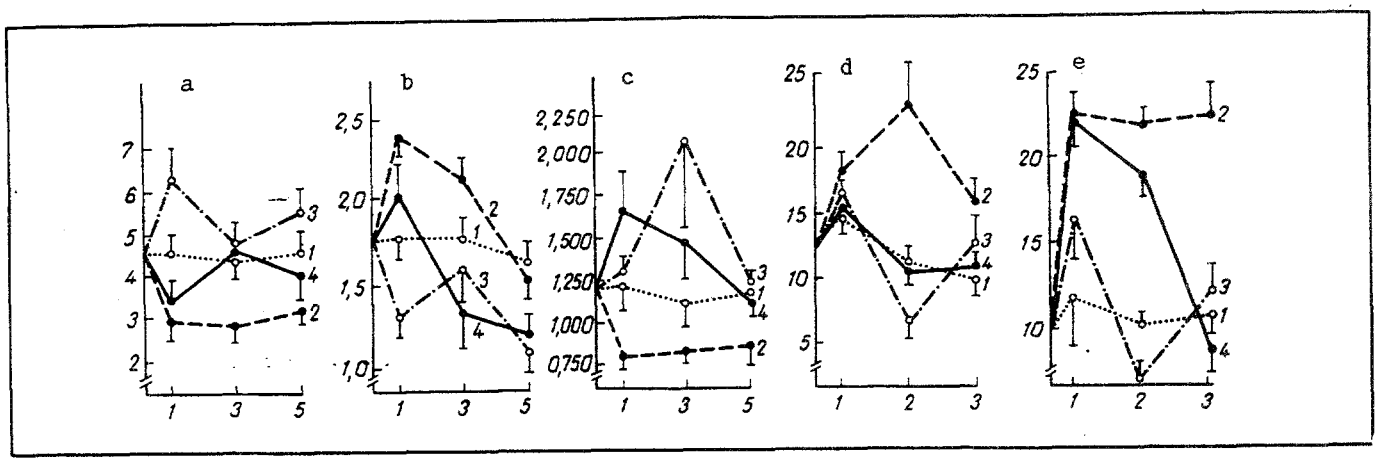

Fig. 1. Effect of dalargin and naloxone on xanthine oxidase (a) and glutathione-S-transferase activity (b), lipid peroxidation (c), and histidase (d) and urocaninase (e) activity in liver tissue. Abscissa, hours after injection. Ordinate: a) reduced ferricytochrome, nmoles/mg protein; b) conjugate of glutathione and chlorodinitrobenzene, nmoles/mg protein·min; c) MDA, nmoles/mg protein; d, e) urocanic acid, nmoles/mg protein·cdsec: 1) 0.9% NaCl, 2) dalargin, 3) naloxone, 4) dalargin + naloxone.

The aim of this investigation was to determine what changes in the hepatoprotective antioxidative action of dalargin may be observed as a result of simultaneous administration of naloxone.

EXPERIMENTAL METHOD

Experiments were carried out on 144 noninbred male albino rats weighing 200 g, with cholestasis, induced by ligation of the common bile duct. The rats were used in the experiments 24 h after the operation. The experiments took place in the following stages: 1) intraperitoneal injection of dalargin in a dose of 10 μg/kg body weight, 2) intraperitoneal injection of naloxone (Du Pont de Nemours, Germany) in a dose of 100 μg/kg body weight, and 3) injection of dalargin and naloxone in the above-mentioned doses (in this series naloxone was injected 10 min before dalargin). Animals with cholestasis, receiving 0.9% NaCl intraperitoneally, served as the control.

The rats were killed by decapitation 1, 3, and 5 h after injection of the preparations. Activity of xanthine oxidase [10], an enzyme catalyzing the formation of hydrogen peroxide and the superoxide-anion radical, activity of glutathione-S-transferase, an enzyme of antioxidative protection [13], and the level of lipid peroxidation [12], based on the degree of change of the malonic dialdehyde (MDA) level, were determined in liver tissue.

Activity of the hepatospecific enzymes histidase and urocaninase in the liver tissue and blood serum was determined.

The protein concentration in the liver tissue was determined by the method in [11]. In a separate series of experiments the effect of dalargin was studied on the Leu-enkephalin level in liver tissue. Leu-enkephalin was determined with the aid of RIA kits (Instar Corporation, USA). Radioactivity was counted on a gamma-counter (Tracor Analytic, USA). In this series dalargin was injected in a dose of 50 μg/kg and the rats were killed 1 h after injection of the preparation.

EXPERIMENTAL RESULTS

It follows from the data in Fig. la that in rats with cholestasis dalargin reduces xanthine oxidase activity in the liver tissue at all times of investigation (by 37.6, 34.8, and 32.5%, $p < 0.01$) after 1, 3, and 5 h respectively. Injection of naloxone led after 1 h to an increase in activity of the enzyme by 38% ($p < 0.02$). At the remaining times of investigation no significant changes in xanthine oxidase activity were found compared with the control data, although 5 h after injection a tendency for the activity of this enzyme to increase still remained.

In response to combined administration of dalargin and naloxone a slightly reduced level of xanthine oxidase activity was observed (although the decrease was less marked than after dalargin alone) 1 h after injection. At other times the parameters were virtually indistinguishable from the control.

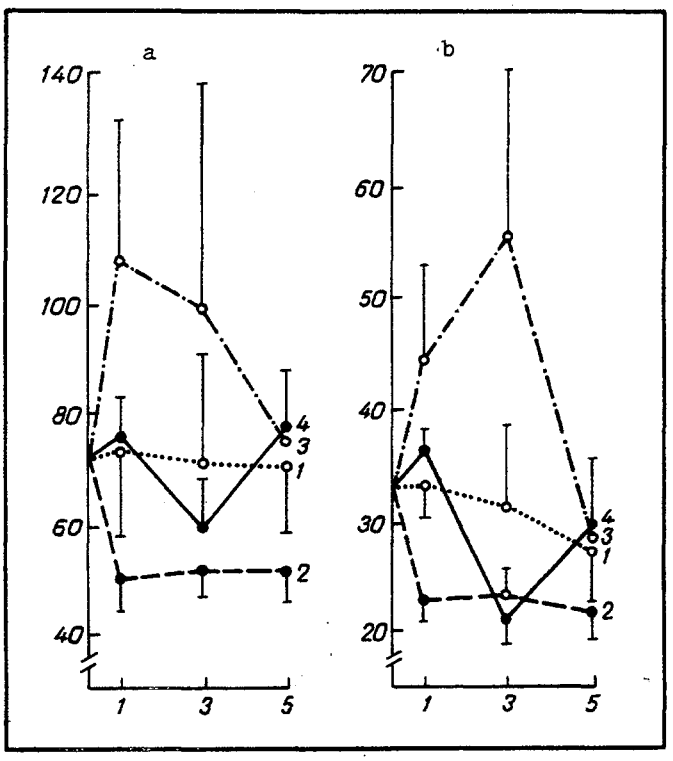

Fig. 2. Effect of dalargin and naloxone on serum histidase (a) and urocaninase (b) activity. Ordinate, urocanic acid, pmoles/ml ·sec. Remainder of legend as to Fig. 1.

Investigation of glutathione-S-transferase activity (Fig. 1b) showed the opposite relationship: injection of dalargin increased activity of the enzyme by 38.0% ($p < 0.001$), the increased activity (by 21.8%, $p < 0.05$) was preserved 3 h after injection, and the return to control values did not occur until after 5 h. Naloxone lowered activity of the enzyme by 25.9% ($p < 0.02$) 1 h after injection, and 3 and 5 h after injection activity of glutathione-S-transferase still remained lowered (by 27.6%, $p < 0.01$). Combined injection of dalargin and naloxone did not lead to any marked changes in activity of the enzyme 1 h after injection, but lowered it after 3 and 5 h (by 36.8 and 26.4%, $p < 0.02$, respectively) compared with the corresponding values determined in animals receiving dalargin alone.

It follows from the data given in Fig. 1c that dalargin significantly lowered the MDA level in the liver tissue 1 and 3 h after injection (by 35.7 and 29.0%, $p < 0.05$, respectively). The MDA level remained low 5 h after injection of dalargin. Injection of naloxone did not change the MDA concentration 1 and 5 h after injection, whereas after 3 h a tendency was observed for it to rise.

On combined injection of dalargin and naloxone the MDA level rose significantly higher than after injection of dalargin alone (by 109.2, by 80.7, and by 25.7%, $p < 0.05$, 1, 3, and 5 h after injection respectively).

The experiments showed (Fig. 2a, b) that under conditions of cholestasis, after injection of dalargin there was a tendency for the release of hepatospecific enzymes histidase and urocaninase into the blood stream to be reduced.

The serum level of activity of these two enzymes 1 and 3 h after injection of naloxone was increased. Combined injection of dalargin and naloxone led to an increase in histidase release into the blood stream, and this was particularly marked 1 and 5 h after injection of the preparations (by 45.8 and 49.4%, $p < 0.02$, respectively), while the urocaninase level was increased 1 h after injection (by 58.5%, $p < 0.001$) compared with values obtained after injection of dalargin alone.

The study of histidase and urocaninase activity in the liver tissue during cholestasis (Fig. 1d, e) showed that injection of dalargin led to an increase in histidase activity by 104.3 and 56.3% ($p < 0.02$) 3 and 5 h after injection respectively, whereas urocaninase activity increased by 88.2, 107.5, and 108.3% after 1, 3 and 5 h respectively.

Injection of naloxone did not significantly change activity of the hepatospecific enzymes 1 and 5 h after injection, but lowered histidase (by 43.8%, $p < 0.02$) and urocaninase (by 37.4%, $p < 0.001$) activity 3 h after injection.

Dalargin combined with naloxone significantly lowered histidase activity in the liver tissue to the level of the control values at all times of investigation, and urocaninase activity 5 h after injection of the preparations.

It thus follows from the above data that treatment with naloxone followed by injection of dalargin greatly reduces the antioxidative effect of the neuropeptide.

Since it seems unlikely that dalargin, which undergoes rapid decomposition in the body through the action of proteinases, could affect metabolic processes a few hours after injection, in order to determine to what extent the metabolic effect of the preparation is realized indirectly through a chain of neurochemical reactions initiated by it, the content of Leu-enkephalin in the liver tissue was studied in animals with cholestasis 1 h after injection of dalargin. It was found that dalargin causes a sharp rise (by 262.3%, $p < 0.05$) of the Leuenkephalin level: the Leu-enkephalin concentration in the control animals was 21.8 ± 11.54 pg/mg tissue, whereas in animals receiving dalargin it was 79.0 ± 19.23 pg/mg tissue.

It can be postulated that the antioxidative effect of dalargin on the liver can be explained on the grounds that dalargin, being a structural analog of Leu-enkephalin and competing with it, displaces the latter from the corresponding tissue depots, leading to the accumulation of Leu-enkephalin in the liver tissue. The final antioxidative effect will be identical in principle in both cases.

Since the opioid antagonist naloxone binds competitively with the opiate receptors of nerve tissue, preventing the action of opioids, the considerable decrease which we found in the antioxidative effect of dalargin on the liver, when injected together with injection of naloxone, suggests that the hepatoprotective effect of this neuropeptide is opiate in character and is effected indirectly through receptor-mediated mechanisms of nerve endings reaching the liver

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