Effects of acetysal, dexamethasone and their combination on drug metabolizing enzyme systems in rat liver microsomes

L.P. TANTCHEVA, D.S. RANGELOVA and Tz.S. STOYTCHEV

Department of Drug Toxicology, Institute of Physiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

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SUMMARY

After 4 days of acetysal treatment (160 mg/kg body weight orally), the following were established: a higher acute toxicity of acetysal, an inducing effect on amidopyrin N-demethylase and analgin N-demethylase activity and increases in cytochrome P-450 and cytochrome b5 content. Aniline hydroxylase activity decreased, thiopental sleeping time was prolonged and UDP-glucuronyltransferase activity was not changed. Dexamethasone, at a dose of 5 mg/kg body weight p.o. for 4 days, did not change acetysal acute toxicity but at a dose of 100 mg/kg i.p. increased it. Thiopental sleeping time was shortened by dexamethasone (100 mg/kg i.p.) but was not changed by dexamethasone at 5 mg/kg p.o., alone or in combination. Dexamethasone at 5 mg/kg increased analgin N-demethylase and UDP-glucuronyltransferase activities, did not change cytochrome P-450 content and decreased aniline hydroxylase activity. The combination with 5 mg/kg dexamethasone increased the activity of amidopyrin N-demethylase, analgin N-demethylase and UDP-glucuronyltransferase and decreased those of amitriptyllin N-demethylase and aniline hydroxylase and cytochrome P-450 content. Ethylmorphine N-demethylase, benzphetamine N-demethylase, NADPH-cytochrome c reductase and glutathione S-transferase activities were not affected significantly by acetysal, dexamethasone or their combination. Hepatic carboxyl esterase was depressed by dexamethasone (5 mg/kg) and was increased by the combination. Lipid peroxidation was not changed by dexamethasone (5 mg/kg) but was decreased by acetysal and the combination.

INTRODUCTION

Glucocorticoids are among the most widely used drugs possessing a multiformal spectrum of physiological and pharmacological effects which make them very useful for treatment of allergic diseases, shock and other extreme situations in medical practice. Now, natural and synthetic glucocorticoids are the drugs of choice in therapy of rheumatism, arthritis, cancer, hormonal, allergic and autoimmune diseases. They are applied at different doses and duration, alone or in combination with other drugs of different pharmacological groups. Their catatotic, syntoxic and permissive effects are well known (1–3). The ability of glucocorticoids to change the reactivity of the organism to other drugs, in order to modulate the drug effects is clearly documented (2–10). Different pharmacokinetic or pharmacodynamic mechanisms of interaction underline the effect of glucocorticoids (2–8,10).
Dexamethasone (DEX) is a synthetic glucocorticoid having a strong enzyme-inducing effect on drug metabolism, stimulating the synthesis of specific cytochrome P-450 isozymes (P-450-DEX or P-450-PCN) (7,11-14).

There are clinical data regarding the unfavourable effects of the combination of glucocorticoids and acetysal, but the mechanisms of their interactions are not yet understood. Acetysal (acetylsalicylic acid, ASA), alone or in combination with analgesic drugs, is widely used in medical practice. ASA administered together with other drugs (anticoagulants, barbiturates, methotrexate, anticonvulsives, antidiabetics, indomethacin, etc.) alters their effects and toxicity (15-19). From the theoretical and practical point of view, it is of interest to try to understand the mechanisms of the glucocorticoid-ASA interaction.

The aim of the present work was to study the effects of 4 day administration of acetysal, DEX and their combination on drug metabolism and lipid peroxidation in liver rat microsomes.

MATERIALS AND METHODS

The experiments were carried out on male Wistar rats (60-180g) divided into 4 groups: group 1, controls; group 2, ASA-treated rats (160 mg/kg body weight, orally); group 3, DEX-treated rats (5 mg/kg body weight, orally); group 4 ASA + DEX treated rats. The animals were treated for 4 consecutive days. Acetysal (acidum acetylsalicylicum, Pharmachim, Bulgaria) and DEX (Prednisolon F, Pharmachim, Bulgaria) were administered in the form of solutions prepared in distilled water with some drops of Tween 80. The effect of DEX at an oral dose of 5 mg/kg body weight, on thiopental (TP) sleeping time and acetysal toxicity was compared to its effect when injected intraperitoneally (i.p.) at a dose of 100 mg/kg body weight.

The control group received an equivalent volume of distilled water with some drops of Tween 80. The solutions were applied through a probe at least 2 h before being deprived of food. At 24 h after the last administration of the compounds or their combination, the following were studied.

Acute acetysal toxicity

A dose of 1600 mg/kg body weight orally, the LD50 of acetysal according to Goldenthal (20), was applied to the four groups of animals. The mortality in each group was registered up to the 14th day after the toxic dose of ASA.

TP sleeping time

Thiopental-Na salt at a dose of 24 mg/kg body weight was injected i.p. and the duration of TP sleeping time in minutes was measured by the loss and restoration of the turning reflex.

Drug metabolizing enzyme activity

At 24 h after the last intake of the compounds or their combination the rats were decapitated. The livers were perfused with 1.15% ice-cold solution of KCl and the microsomal resp. cytosolic fraction was isolated by differential centrifugation.

The following parameters were studied in microsomes: N-demethylation of the substrates ethylmorphine (EMND), amidopyrine (aminophenazonum) (APND), benzphetamine (BPND), analgin (noramidopyrinimetad sulfonas natricum) (ANND) and amitryptyl line (AMIND); determining the HCHO formed (21); aniline hydroxylase (AH) activity (22); activity of NADPH cytochrome c-reductase (CCR) (23); cytochrome P-450 (cyt P-450) and cytochrome bs (cyt bs) content (24); activity of liver carboxylesterase (25) (diethyl-p-nitrophenyl phosphate as substrate); and UDP glucuronyl transferase (UDP-GTF) activity (26) (with p-nitrophenol as substrate).

Glutathione S-transferase (GSTF) activity (27) was determined in the cytosolic fraction using 1-chloro-2,4-dinitrobenzene as substrate and in microsomes lipid peroxidation by the thiobarbituric acid (TBA) test of Buege and Aust (28). The amount of malondialdehyde (MDA) was registered using a molar extinction coefficient of 1.56 x 10-5 M^-1 cm^-1. The enzyme activity was expressed in nmol product formed by 1 mg protein for 1 min. Microsomal cyt P-450 and cyt bs contents were calculated in nmol/mg protein. Protein content was determined by the method of Lowry et al. (29). The data were statistically analyzed by Student’s t-test and the means ± SEM are presented.

RESULTS

Acetysal toxicity

The acute oral toxicity of ASA increased after its 4 day administration (4/6 rats died vs 2/6 controls). DEX, at a dose of 5 mg/kg body weight, did not change ASA toxicity as compared to the controls (2/6), but at a dose of 100 mg/kg body weight this