REFERENCES

1. Webster LT, Drugs used in the chemotherapy of protozoal infections. In: *The Pharmacological Basis of Therapeutics* (Eds. Gilman AG, Goodman LS, Rall TW and Murad F), pp. 1029–1048. Macmillan, New York, 1985.
2. Alving AS, Eichelberger L, Craige B Jr, Jones R Jr, Whorton CM and Pullman TN, Studies on the chronic toxicity of chloroquine (SN-7618). *J Clin Invest* **27**: 60–65, 1948.
3. Crouzetto J, Vicaut E, Palombo S, Girro C and Fournier PE, Experimental assessment of the protective activity of diazepam on the acute toxicity of chloroquine. *J Toxicol Clin Toxicol* **20**: 271–279, 1983.
4. Riou B, Barriot P, Timailto A and Baud FJ, Treatment of severe chloroquine poisoning. *N Engl J Med* **318**: 1–6, 1988.
5. Clark JA, Liu L, Price M, Hersh B, Edelson M and Pasternak GW, κ Receptor multiplicity: Evidence for two U50,488-sensitive κ_1 subtypes and a novel κ_3 subtype. *J Pharmacol Exp Ther* **251**: 461–468, 1989.
6. Gavish M, Weizman A, Youdim MBH and Okun F, Regulation of central and peripheral benzodiazepine receptors in progesterone-treated rats. *Brain Res* **409**: 386–390, 1987.
7. Gavish M, Awad M and Fares F, Existence of sites for anions and divalent cations in the solubilized γ-aminobutyric acid/benzodiazepine receptor complex. *J Neurochem* **45**: 760–765, 1985.
8. Clark JA, Houghten R and Pasternak GW, Opiate binding in calf thalamic membranes: A selective μ_1 binding assay. *Mol Pharmacol* **34**: 308–317, 1988.
9. VonVoightlander PF, Lahti RA and Ludens JH, U-50,488: A selective and structurally novel non-*mu* (kappa) opioid agonist. *J Pharmacol Exp Ther* **224**: 7–12, 1983.
10. Cheng Y-C and Prusoff WH, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol* **22**: 3099–3108, 1973.
11. Chou T-C, Relationships between inhibition constants and fractional inhibition in enzyme-catalyzed reactions with different numbers of reactants, different reaction mechanisms, and different types and mechanisms of inhibition. *Mol Pharmacol* **10**: 235–247, 1974.
12. Djelardje S, *Intoxications Volontaires par la Chloroquine*. M.D. dissertation, Medical University of Abidjan, Ivory Coast, 1976.
13. Bondurand A, N'Dri K, Coffi S and Saracino E, L'Intoxication a la chloroquine au Centre Hopitalo-Universitaire d'Abidjan. *Afr Med* **179**: 239–242, 1980.
14. Champagne D, *Intoxication Volontaires a la Chloroquine*. M.D. dissertation, Medical University of Paris, France, 1973.
15. Tallman JF, Thomas JW and Gallager DW, GABAergic modulation of benzodiazepine binding site sensitivity. *Nature* **274**: 383–385, 1978.
16. Weissman BA, Bolger GT, Isaac L, Paul SM and Skolnick P, Characterization of the binding of [^{3}H]Ro 5-4864, a convulsant benzodiazepine, to guinea pig brain. *J Neurochem* **42**: 969–975, 1984.
17. Pasternak GW, Multiple morphine and enkephalin receptors and the relief of pain. *J Am Med Assoc* **259**: 1362–1367, 1988.
18. Snead OC III, Opiate-induced seizures: A study of μ- and δ-specific mechanisms. *Exp Neurol* **93**: 348–358, 1986.

Biochemical Pharmacology, Vol. 41, No. 10, pp. 1536–1537, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Protection by boswellic acids against galactosamine/endotoxin-induced hepatitis in mice

(*Received* 20 *September* 1990; *accepted* 4 *January* 1991)

In the preceding communication of this series we reported that crude ethanolic extracts of the gum resin exudate from *Boswellia serrata* [1] inhibited by leukotriene B_4 (LTB_4) production from endogenous arachidonic acid by rat peritoneal polymorphonuclear leukocytes *in vitro*, most likely by decreasing the activity of the 5-lipoxygenase (5-LO). We identified boswellic acids as the active principle (manuscript in preparation). However, it is not clear whether such an effect can also be observed in an *in vivo* animal model after oral administration of the drug.

Recent reports showed that administration of endotoxin to rats led to an increase of leukotriene secretion into the bile [2] and that leukotriene synthesis and action inhibitors exerted protective effects against galactosamine/endotoxin-induced lethality [3] and hepatitis [4] in mice *in vivo*. The liver damage biochemically is evidenced by increases in serum sorbitoldehydrogenase (SDH), aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) activities [4].

In the present study, we investigated the *in vivo* effect of the acetyl-boswellic acids (ac-BA) after oral application on the galactosamine/salmonella endotoxin-induced liver damage in mice. The data indicate that pretreatment by ac-BA 1 hr before the intoxication substantially reduced increased levels of serum enzyme activities.

Materials and Methods

D-Galactosamine HCl (GalN) was purchased from Serva (Heidelberg, F.R.G.), endotoxin (*Salmonella abortus equi* lipolysaccharide) from Sebak (Berlin, F.R.G.) and sodium-heparin from Braun (Melsungen, F.R.G.).

The acetyl-boswellic acids (ac-BA) tested in this study were isolated from the gum resin of *Boswellia serrata* according to Winterstein and Stein [5] and characterized by ^{1}H-NMR, MS, i.r. and UV-spectra (manuscript in preparation). The ac-BA mixture applied in the present study was composed of acetyl-β-BA (95%), acetyl-α-BA (2%) and acetyl-11-keto-β-BA (3%).

Male albino mice (han-NMRI) were purchased from the Zentralinstitut für Versuchstiere (Hannover, F.R.G.) and kept on standard diet (Altromin, Lage, F.R.G.) with free access to food and water under 12 hr dark/light rhythm.

Their average weight was 32 g. GalN/endotoxin-evoked hepatitis was induced according to Wendel and Tiegs [4]. Briefly, tested drugs suspended in tylose or vehicle (1% tylose) were orally applied 1 hr before the intraperitoneal injection of 700 mg/kg GalN and 33 μg/kg salmonella endotoxin as a mixture in phosphate buffered saline. After 8 hr blood was withdrawn from the arteria carotis externa into 2.5% heparin. Serum aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) activities were quantified with commercial kits from Boehringer Mannheim (F.R.G.), and sorbitole dehydrogenase (SDH) according to Gerlach [6]. Data are given as means ± SD. Statistical significance was evaluated according to Student's *t*-test.

Results and Discussion

As demonstrated by the data of Fig. 1A and previously reported by others [4] the intraperitoneal administration of GalN/endotoxin dramatically increased SGOT roughly by 10 times within 8 hr compared to the serum enzyme activity in untreated animals. In parallel, the SGPT activity had risen from 30 to 2520 units/L (Fig. 1B) and the serum SDH from 10 to 1320 units/L (Fig. 1D). When pretreated 1 hr before the application of GalN/endotoxin, ac-BA (1500 mg/kg body wt) substantially and significantly reduced the increase in the activities of SGOT, SGPT and SDH (Fig. 1A–C).

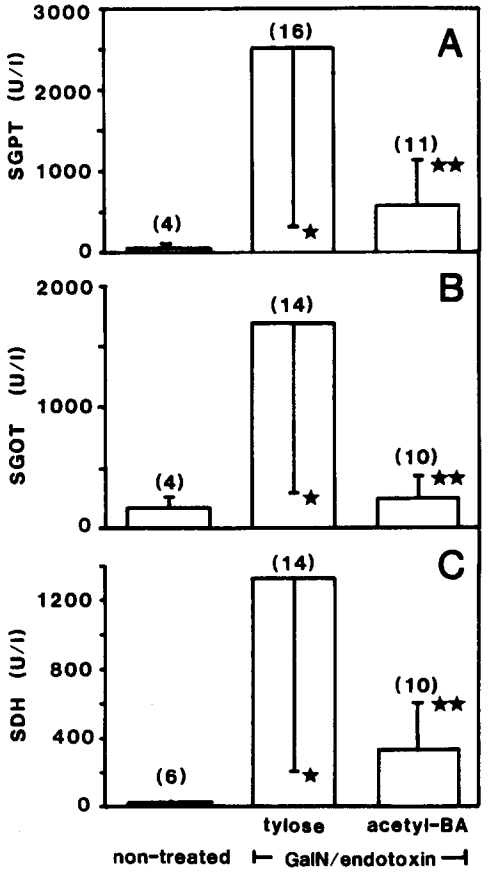

Fig. 1. Protection by oral acetyl-boswellic acids (ac-BA) pretreatment against galactosamine/endotoxin-induced hepatitis in mice: SGPT (A), SGOT (B) and SDH levels (C). Galactosamine (700 mg/kg) and endotoxin (33 μg/kg) were injected intraperitoneally as a mixture in phosphate-buffered saline. Serum enzyme activities were assayed after 8 hr and expressed as units/L (means ± SD; number of observations in parentheses). Ac-BA in tylose or tylose were orally applied 1 hr prior to the galactosamine/endotoxin injection. * $P \leq 0.001$ vs non-treated animals; ** $P \leq 0.001$ vs tylose controls.

It was demonstrated previously that the *in vivo* model used here is sensitive to modulators of eicosanoid metabolism which inhibit the formation of leukotrienes (LT), whereas compounds which preferentially block the cycloxygenase product formation such as aspirin or ibuprofen have no effect on GalN/endotoxin-induced liver injury [2, 4]. It was shown that crude ethanolic extracts of the gum resin of *Boswellia serrata* [1] as well as purified boswellic acids (manuscript in preparation) inhibit *in vitro* the production of LT type mediators of inflammation, most likely by decreasing directly the activity of the key enzyme for the synthesis of LT metabolites from arachidonic acid. Therefore, we suggest that the prevention of the signs of GalN/endotoxin-induced hepatitis by ac-BA might be related to its ability to inhibit the 5-lipoxygenase pathway. In line with this interpretation, established 5-LO inhibitors (i.e. diethylcarbamazine and ebselen) were reported to be effective in this inflammatory model. In conclusion, our findings indicate that the inhibition of leukotriene formation may underlie the hepatoprotective as well as the anti-inflammatory activity of boswellic acids.

In summary, intraperitoneal application of galactosamine and salmonella endotoxin caused acute liver injury in male albino NMRI mice. Within 8 hr serum sorbitoldehydrogenase (SDH), aspartate aminotransferase (SGOT) and alanine aminotransferase (SGPT) activities had risen from 10 to 1330, from 170 to 1700 and from 30 to 2520 units/L, respectively. When perorally given 1 hr before the intoxication with galactosamine/endotoxin acetyl-boswellic acid isolated from the gum resin exudate of *Boswellia serrata* potently and significantly reduced serum enzyme activities. Since it is known that cyclooxygenase pathway inhibitors are not effective in this *in vivo* animal model the protection by acetyl-boswellic acids is interpreted in terms of their recently recognized ability to inhibit the formation of leukotrienes.

Department of Pharmacology
Pharmaceutical Institute
University of Tübingen
Auf der Morgenstelle 8
D-7400 Tübingen
Federal Republic of Germany

H. SAFAYHI
T. MACK
H. P. T. AMMON

REFERENCES

1. Ammon HPT, Mack T, Singh GB and Safayhi H, Inhibition of leukotriene B_4 formation in rat peritoneal neutrophils by an ethanolic extract of the gum resin exudate of *Boswellia serrata*. *Planta Med*, in press.
2. Hagmann W, Denzlinger C and Keppler D, Production of peptide leukotrienes in endotoxin shock. *FEBS Lett* **180**: 309–313, 1985.
3. Hagmann W and Keppler D, Leukotriene antagonists prevent endotoxin lethality. *Naturwiss* **69**: 594–595, 1982.
4. Wendel A and Tiegs G, A novel biologically active seleno-organic compound—VI. Protection by Ebselen (PZ 51) against galactosamine/endotoxin-induced hepatitis in mice. *Biochem Pharmacol* **35**: 2115–2118, 1986.
5. Winterstein A and Stein G, Untersuchungen in der Saponinreihe. Zur Kenntnis der Mono-oxy-triterpensäuren. *Hoppe Seylers Z Physiol Chem* **208**: 9–25, 1932.
6. Gerlach U, Sorbitol dehydrogenase. In: *Methods of Enzymatic Analysis* (Ed. Bergmeyer HU), Vol. III, pp. 112–117. VCH, Weinheim, 1983.