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Danger of bucolome in infants with hyperbilirubinaemia

Experimental evidence

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SUMMARY Effects of bucolome on congenitally jaundiced Gunn rats were examined. Plasma total bilirubin level fell immediately after a single injection of bucolome and the lowered level persisted for more than 6 hours. Plasma unbound-bilirubin level and cerebellar bilirubin content increased simultaneously with the drop in total plasma bilirubin level. Kernicterus was observed in the brains of the treated rats 6 hours after the treatment. LD_{50} of the drug in jaundiced rats was about 37 mg/kg, about one-tenth of the value in nonjaundiced rats. It is suggested that bucolome displaces bilirubin from albumin, transferring bilirubin from blood into tissues including the brain, and resulting in kernicterus. The use of bucolome in infants with hyperbilirubinaemia is inadvisable.

Bucolome (1-cyclohexyl-5-n-barbituric acid), a barbiturate derivative, has been used as an antiphlogistic drug, but after it was found to decrease serum bilirubin levels without the hypnotic effect of barbiturates (Yamamoto and Sakamoto, 1971), it was given to infants for treating or preventing neonatal hyperbilirubinaemia (Baba, 1972/73; Okada *et al.*, 1972; Segni *et al.*, 1977). On the other hand, it was reported that the drug displaced bilirubin from albumin (Yamamoto and Adachi, 1974) and increased the amount of unbound bilirubin *in vitro* (Yamaji *et al.*, 1975). We have studied the *in vivo* effects of the drug on congenitally jaundiced mutant rats, and conclude that there are good reasons why the drug should not be given to infants with hyperbilirubinaemia.

Materials and methods

Homozygous Gunn rats are unable to conjugate bilirubin (Strebel and Odell, 1971) and they show life-long hyperbilirubinaemia, while heterozygotes do not. Homozygous littermates from 3 litters were equally alloted to 4 groups (A–D). Animals were starved for 16 hours beforehand to avoid the effect of free fatty acids on the plasma unbound-bilirubin level (Sato and Semba, 1977). 100 mg/kg body weight of sodium bucolome, dissolved in saline, was injected subcutaneously to sucklings of groups B, C, and D, on the 15th day of life. No bucolome was administered to group A.

In group A, blood was sampled under deep anaesthesia. In groups B, C, and D blood sampling was done 30 minutes, 3 hours, and 6 hours after an injection of bucolome, respectively. After blood sampling and perfusion with saline solution, the brain was removed. The cerebellum was measured for bilirubin content, and the rest of the brain fixed in 10% formalin. Next day the fixed brain was sliced and examined for yellow discoloration under a dissecting microscope. Total plasma bilirubin level, plasma unbound-bilirubin level, and cerebellar bilirubin content were measured by the methods of Malloy and Evelyn (1937), Chunga and Lardinois (1971), and Katoh *et al.* (1975).

In another experiment, 6 doses of sodium bucolome, from 10 mg/kg to 60 mg/kg, were subcutaneously injected to jaundiced Gunn rats on the 15th day of life. To nonjaundiced heterozygous rats, 400 mg/kg of the drug was administered. Their survival rates after 72 hours were examined.

Results

Total plasma bilirubin level decreased to 24% within 30 minutes after bucolome administration and the lowered level persisted for more than 6 hours

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(Fig. 1). Plasma unbound-bilirubin level increased by 353%, this high level persisting for more than 6 hours (Fig. 2). Cerebellar bilirubin content increased to 323%, 501%, and 895% at 30 minutes, 3 hours, and 6 hours respectively (Fig. 3).

On examination of the sliced brains, diffuse yellow staining of the brain was evident at 30 minutes and at 3 hours after the drug injection. At 6 hours, localised yellow staining, kernicterus, was evident in nucleus subthalamicus, some thalamic nuclei, the floor of the IVth ventricle, and nucleus colliculi inferioris.

The effect of bucolome on the survival rates of jaundiced rats is summarised in the Table. None of

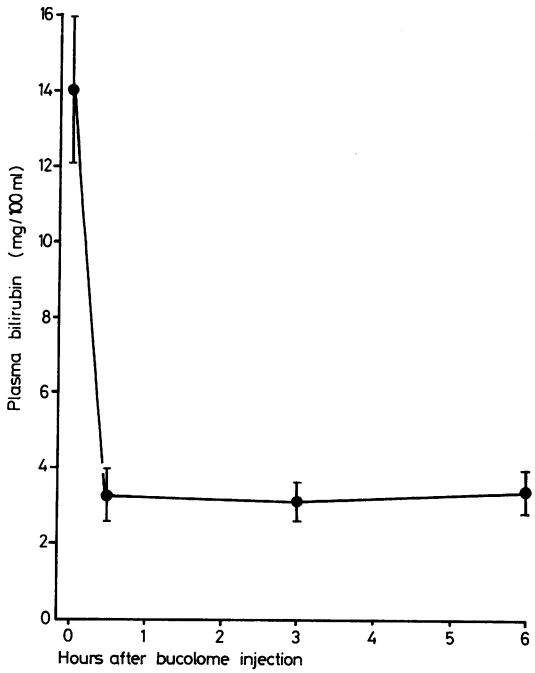

Fig. 1 *Time course of total plasma bilirubin level after an injection of bucolome. The vertical lines in Figs 1–3 indicate SD.*
Conversion: Traditional units to SI—*Bilirubin: 1 mg/100 ml ≈ 17·1 μmol/l.*

Table *Survival rate of jaundiced Gunn rats after subcutaneous injection of bucolome*

Dose of bucolome (mg/kg)	*No. of rats examined*	*Survived (%)*
0 (vehicle)	6	6 (100)
10	6	6 (100)
20	8	7 (88)
30	8	4 (50)
40	8	3 (38)
50	13	4 (31)
60	4	0 (0)

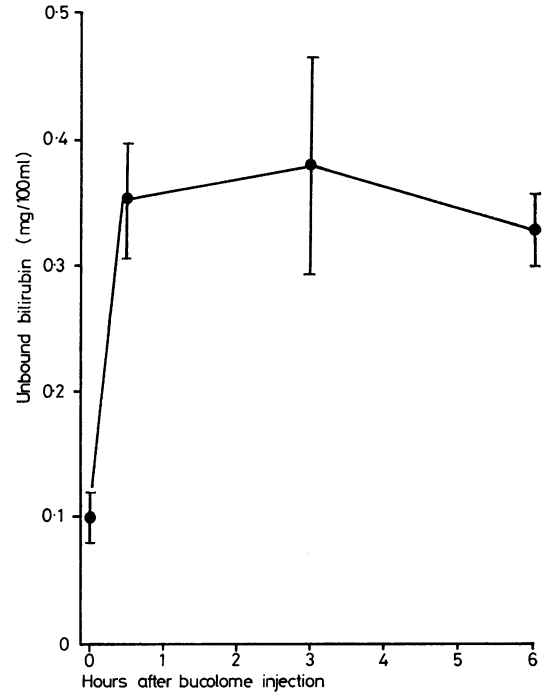

Fig. 2 *Time course of plasma unbound-bilirubin level after an injection of bucolome.*

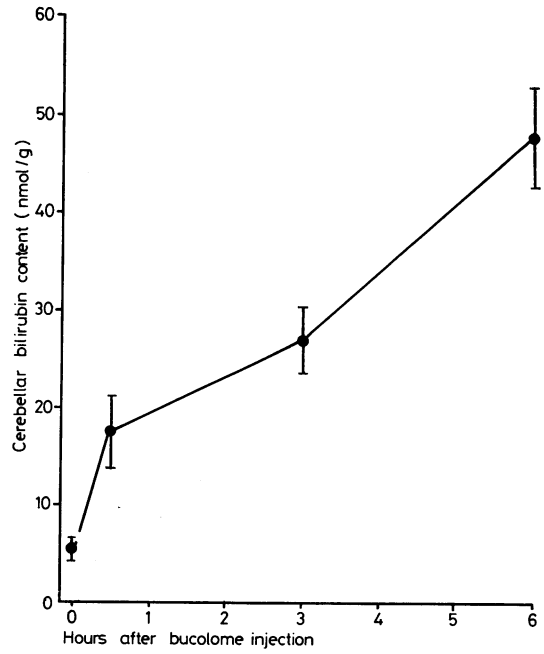

Fig. 3 *Time course of cerebellar bilirubin content after an injection of bucolome.*

the 6 rats which received 10 mg/kg bucolome died, while 13 of the 17 rats which received more than 50 mg/kg died. In the jaundiced rats on their 15th day of life LD_{50} was calculated as 37 mg/kg body weight. On the other hand, in heterozygotes, 2 out of 5 rats receiving 400 mg/kg of the drug survived for more than 72 hours, and no kernicterus was observed in any.

Discussion

A rapid fall in plasma total bilirubin level after bucolome administration was observed, coincidentally with the report of Yamamoto and Adachi (1974), in homozygous Gunn rats. It has been suggested that bucolome induces bilirubin-conjugating enzymes in the liver as phenobarbitone has been shown to do (Yamamoto and Sakamoto, 1971). In the present study, a large decrease of plasma total bilirubin level occurred within 30 minutes; this is too short for the induction of bilirubin-conjugating enzymes to operate. In addition, phenobarbitone is not an effective inducer of bilirubin-conjugating enzymes in homozygous Gunn rats (Robinson *et al.*, 1971). Thus other mechanisms are likely to be responsible for the plasma bilirubin lowering effect of bucolome.

Yamaji *et al.* (1975) reported that bucolome increased unbound bilirubin levels *in vitro*, and the same effect was seen in the present *in vivo* experiments. Although the plasma unbound-bilirubin level does not necessarily indicate the risk of kernicterus (Sato and Semba, 1978), the rapid and persistent increase of unbound-bilirubin was accompanied by a rapid initial increase and a further gradual increase of the bilirubin content of the cerebellum, which was followed by kernicterus after several hours. It is suggested that bucolome displaces albumin-bound bilirubin and so leads to kernicterus.

In Wistar rats, LD_{50} of bucolome is around 400 mg/kg (T. Matsuzawa, personal communication). In agreement with this, 2 among the 5 heterozygotes receiving 400 mg/kg bucolome on the 15th day of life survived. In *jaundiced* rats, LD_{50} was only 37 mg/kg. Bucolome was thus specifically toxic to jaundiced animals, and the cause of death was shown at necropsy to be kernicterus. Displacement of bilirubin from albumin-binding by bucolome may explain the specific toxicity of bucolome in the jaundiced animals.

Sulphafurazole has been observed to precipitate kernicterus in preterm infants (Silverman *et al.*, 1956). Bucolome might well cause kernicterus in jaundiced infants as in jaundiced rats. Although some 90 babies have received bucolome (Baba, 1972/73; Okada *et al.*, 1972; Segni *et al.*, 1977) without reports of death or severe neurological defects, follow-up of the treated infants is essential.

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