application of colchicine to the nerve leads to a decrease in the number of Merkel cells too (fig. 1). Thus 30 days after colchicine application to the nerve, the number of Merkel cells was 55% of control ($p < 0.05$). The structure of vibrissae on the side where saline solution was applied to the nerve was compared with the structure of vibrissae on the contralateral side. There were no statistically significant differences in the number of Merkel cells in these 2 groups of vibrissae. The ultrastructural examination of the remaining Merkel cells revealed the following alterations 30, 45 and 60 days after the nerve transection: a greater density of the cytoplasm and nucleus, an increment in number and size of the vacuols and greater heterogenity in the density of specific granules. These ultrastructural changes in Merkel cells were accompanied by Wallerian degeneration of nerve terminals in the Merkel cell-neurite complexes. At 45 and 60 days after nerve transection numerous nerve fibers appear again in the connective tissue of vibrissae beneath glassy membrane. 30, 45 and 60 days following colchicine nerve treatment, ultrastructural examination of ipsilateral vibrissae revealed signs of morphological modifications in the remaining Merkel cells (fig. 3). Their ultrastructure was similar to that observed after the nerve transection. However, the contacts between Merkel cells and nerve terminals were always observed after colchicine nerve treatment. Some Merkel cell-neurite complexes showed normal morphology. On the other hand no structural alterations were detected in the contralateral vibrissae in the cases either of nerve transection and colchicine nerve treatment, nor were alterations detected in the ipsilateral vibrissae of cats whose nerves were treated with saline solution alone. No changes in the number and structure of keratinocytes could be observed in vibrissae as a result of the experiments.

It has been reported that denervation of tactile corpuscles in cats was followed by the loss of Merkel cells[9,10]. Authors believe that the effect does not appear to be the result of the generalized skin alterations. It seems to be most likely that sensory innervation of the epithelium is requisite for maintaining Merkel cell integrity. This supposition was confirmed by the observations on tactile corpuscles of cat skin which indicated a close connection between Merkel cells' regeneration and the reappearance of sensory nerve fibers in the epithelium[9,11]. Consequently, the decrease in number of Merkel cells after nerve transection is caused by the interruption of the trophic influence of nerves on the epithelium. The nature of nerve influence on the target (inducing and/or supporting differentiation) in the case of Merkel cells is still unknown. It is highly probable that blockade of axonal transport with colchicine prevents the secretion of chemical factors which can be released from the nerve terminals and provide control of Merkel cells integrity. The nature of these factors remains to be demonstrated.

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The protective effect of polyriboinosinic acid-polyribocytidylic acid against the occurrence of galactosamine-induced liver cell injury in rat[1]

S. Sakakibara, Y. Shibayama, I. Ueda and H. Kagamiyama

Department of Medical Chemistry, and First Department of Pathology, Osaka Medical College, Daigaku-cho 2-7, Takatsuki City, Osaka 569 (Japan), April 20, 1982

Summary. A marked increase of serum transaminase activities, histological changes of livers similar to those seen in viral hepatitis in man, and inhibition of hepatic protein synthesis were observed in rats following a single injection of D-galactosamine-HCl. These galactosamine-induced phenomena were prevented by the pretreatment of polyriboinosinic acid-polyribocytidylic acid 24 h before the galactosamine administration.

The administration of D-galactosamine (GalN) to rats has been known to induce alterations in livers which closely resemble human viral hepatitis[2-4]. It is interesting to note that GalN-induced liver cell injury is different in its histological features from other experimental hepatitis caused by various hepatotoxic chemicals. Such observations suggest that a similar mechanism may be involved in producing liver cell lesions in GalN-induced hepatitis and human viral hepatitis. Interferon or interferon-inducing agents have been used for therapy or protection from viral infection. Since it has been recently reported that interferon has a therapeutic effect against human viral hepatitis[5,6], we are interested in the effect of these agents on GalN hepatitis.

In the present report we will describe the protective effect of polyriboinosinic acid-polyribocytidylic acid (poly IC), which has been known to induce interferon in vivo and in vitro[7], against hepatocyte damage caused by GalN.

Materials and methods. Wistar strain male rats weighing 180–200 g were used and fed laboratory chow and water ad libitum. GalN-HCl and poly IC were purchased from Sigma Chemical. [U-^{14}C]-amino acid mixture (10 mCi/mmole) was obtained form Radiochemical Centre, Amersham.

All reagents used for injections were dissolved in physiological saline solution, neutralized immediately before use and given by a single i.p. injection. Control animals received an

equivalent amount of 0.9% NaCl solution. Blood was collected from the inferior vena cava and livers were taken out under ether anesthesia.

Serum GOT (glutamic-oxaloacetic transaminase EC 2.6.1.1.) and GPT (glutamic-pyruvic transaminase EC 2.6.1.2.) were assayed by the method of Karmen[8] using the test kits of Centrifichem (Baker Instrument Corp.). Enzyme activities were expressed in mIU/ml serum (37 °C). Livers for a light microscopic examination were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin-eosin. For measuring the rate of protein synthesis in rat liver, each animal received 5 μCi of [U-^{14}C]-amino acid mixture 3 h after a GalN or physiological saline administration. After 20 min, livers were excised and the incorporated radioactivity in the total liver protein was determined by the method of Anukarahanonta et al.[9]. The amount of protein was determined by the method of Lowry et al.[10].

Results and discussion. It has been established that hepatic enzyme leakage and histopathological changes of livers are apparent about 24 h after the single injection of GalN[3,4]. Therefore, blood and livers were collected 24 h after the GalN injection and serum GOT and GPT activities and histological changes of livers were examined. As shown in table 1, serum GOT and GPT activities were increased markedly by the administration of GalN (400 mg/kg b.wt), but poly IC (10 mg/kg b.wt), given 24 h prior to GalN, caused a pronounced protective effect against the GalN-induced increase of transaminase activities. Table 1 also shows that an injection of 10 mg/kg of poly IC itself did not exhibit any significant influence on serum transaminases. These results were correlated to the findings of histological examination of livers (fig.). Compared with a normal rat liver (fig. A), the livers of rats 24 h after the GalN administration showed typical features of acute severe viral hepatitis as shown in figure B, in agreement with those described by Keppler et al.[2-4]. Foci of hepatocellular necrosis with inflammatory cell infiltration, acidophilic degeneration and acidophilic bodies were disseminated throughout the lobules. However, the administration of poly IC 24 h prior to the GalN treatment prevented markedly the development of morphological changes by GalN as shown in figure C. No focus of hepatocellular necrosis was found, while very few, if any, acidophilic bodies were observed. Improvement of liver cell damage was not observed when poly IC was given simultaneously or 2 h after the administration of GalN (data not shown).

Table 1. The effect of poly IC pretreatment on serum transaminase activities of GalN-administered rats

Poly IC (10 mg/kg)	GalN (400 mg/kg)	GOT (mIU/ml)	GPT (mIU/ml)
−	−	34 ± 3	18 ± 2
−	+	690 ± 267[a]	338 ± 118[a]
+	+	68 ± 24[b]	40 ± 8[b]
+	−	42 ± 5	14 ± 3

Each value represents mean ± SD of 5 rats. Significantly different from control rats (a) and from GalN-administered rats (b), $p < 0.001$.

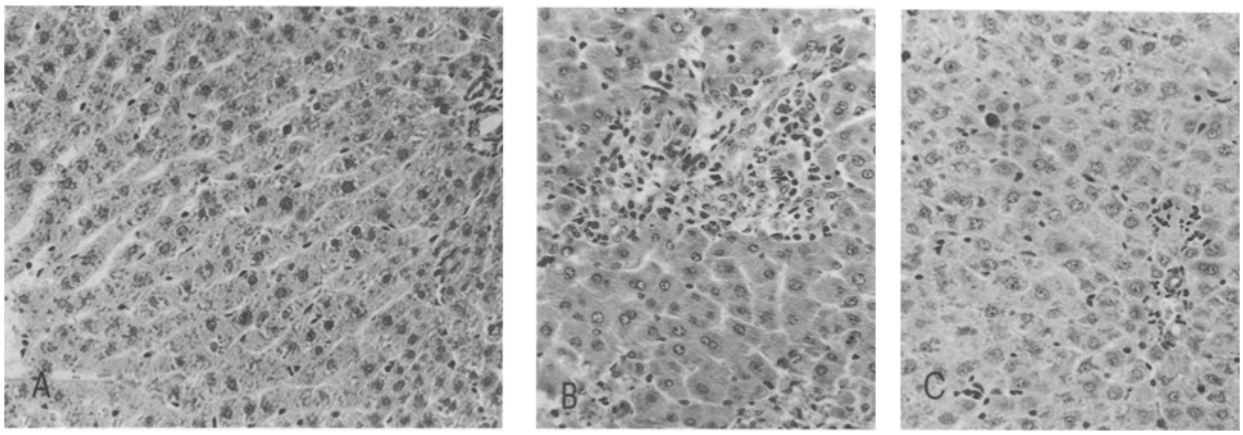
Table 2. Prevention of the GalN-induced inhibition of hepatic protein synthesis by poly IC preadministration

Poly IC (10 mg/kg)	GalN (400 mg/kg)	Incorporated radioactivity (dpm/mg liver protein)
−	−	260 ± 47
−	+	122 ± 47[a]
+	+	248 ± 56
+	−	268 ± 50

Values represent mean ± SD of 6 rats. [a]Significantly different from the value of control rats; $p < 0.001$.

In addition to the above experiments, an effect of poly IC pretreatment on hepatic protein synthesis was also examined. Anukarahanonta et al.[9] reported that GalN inhibited hepatic protein synthesis. They observed that the maximum inhibition was reached within 2 h and remained for several hours after the GalN administration. As shown in table 2, the incorporation of labeled amino acids into total protein of rat liver was inhibited 50–60% in rats 3 h after the GalN administration. However, when poly IC was given 24 h prior to GalN, the inhibition of protein synthesis was not observed.

These results suggest that poly IC prevents the occurrence of GalN-induced hepatocyte damage. Although poly IC has been known to be an interferon inducer, the conclusion, that the protective effect of poly IC against GalN-induced hepatocyte damage resulted from its interferon induction, is not drawn because poly IC has also been reported to have some other pharmacological effects[11]. Studies to elucidate the mechanism of poly IC action and to know whether this effect is restricted only to poly IC or is common to other interferon inducing agents are now underway.



The effect of poly IC preadministration on the histology of the rat livers given GalN (H&E, × 200). *A* Normal liver, *B* the liver of the rat 24 h after the administration of GalN (400 mg/kg b.wt), *C* the liver of the rat given poly IC (10 mg/kg b.wt) 24 h prior to the GalN administration.

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Prolongation by selenium of pentobarbital hypnosis in the male rat

R.C. Schnell and J.L. Early

Department of Pharmacodynamics and Toxicology, College of Pharmacy, University of Nebraska Medical Center, Omaha (Nebraska 68105, USA), and Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette (Indiana 47907, USA), April 15, 1982

Summary. Following treatment with sodium selenite, pentobarbital hypnosis was prolonged in male rats. The maximal effect occurred at 3–4 days following selenium treatment and the threshold dose for the effect was found to be 2.4 mg Se/kg (i.p.).

Selenium is now recognized as an essential trace element constituting an integral portion of the enzyme, glutathione peroxidase[2,3]. Until approximately 25 years ago, selenium was of concern primarily because of its toxicity. The toxicity induced by selenium exhibits many manifestations and depends upon many factors such as age, dietary constituents, species, and sex[4–6]. Toxicity of selenium can be induced acutely in laboratory animals or can occur following chronic exposure as is generally seen in farm animals eating a sufficient quantity of highly seleniferous plants[6].

The administration of many other metals, such as lead[7], cadmium[8], and manganese[9] produce hepatic effects such as inhibition of drug biotransformation which can lead to an altered responsiveness to other drugs including the barbiturates. This study was undertaken to examine the effect of acute selenium administration on drug response in the male rat.

Methods. Male, Sprague-Dawley rats weighing 160–240 g were obtained from Laboratory Supply Co. (Indianapolis, IN) and housed in community cages for at least 1 week prior to use. Animals were maintained in environmentally controlled rooms at approximately 22 °C under a 12-h alternating light-dark cycle (L) (06.00–18.00 h) with free access to laboratory food (Wayne Lab. Blox, Allied Mills, Inc., Chicago, IL) and tap water.

Sodium selenite (Na_2SeO_3) and pentobarbital Na solutions for injections were prepared using distilled, deionized water such that each animal received 1 ml/kg b.w, i.p. The duration of hypnosis was defined as the time elapsing from loss to recovery of the righting reflex. Statistical analyses were performed using analysis of variance (ANOVA) followed by Dunnett's test where appropriate. The acceptable level of significance was established as $p < 0.05$.

Results. In the first experiment the duration of hypnosis following the administration of pentobarbital (40 mg/kg, i.p.) to male rats treated 72 h prior to a challenge with selenium in doses of 0.8, 1.6, or 2.4 mg/kg was determined. These doses were chosen since they had been used by

Table 1. Dose-response of selenium prolongation of pentobarbital hypnosis in male rats

Selenium dose (mg/kg)	Duration of hypnosis (min ± SEM)
0	95 ± 9
0.8	97 ± 9
1.6	99 ± 7
2.4	150 ± 24*

Rats (8 per group) received pentobarbital (40 mg/kg, i.p.) 72 h after treatment with selenium in the designated doses and duration of hypnosis was determined. * Significantly different from control values ($p < 0.05$).

Table 2. Time-course of selenium prolongation of pentobarbital hypnosis in male rats

Time after selenium (h)	Duration of hypnosis (min ± SEM)
Controls	76 ± 7
1	66 ± 13
6	96 ± 16
12	78 ± 5
24	84 ± 7
48	116 ± 28
72	132 ± 20*
96	138 ± 11*

Rats (5–7 per group) received Na selenite (2.4 mg Se/kg) and at the specified time intervals received pentobarbital (40 mg/kg). The duration of hypnosis was measured. Controls received saline (1 mg/kg) 4 days prior to pentobarbital. * Significantly different from control values ($p < 0.05$).