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CASE STUDY OF CARCINOGENICITY BY
INITIATION-PROMOTION MODEL

Mamoru MUTAI and Toyohiko AOKI*

*Toxicology Laboratory, Yokohama Research Center, Mitsubishi Chemical Corporation,
1000 Kamoshda-cho, Aoba-ku, Yokohama 227, Japan***Drug Safety Research Laboratories, Eisai Co., Ltd.,
Kawashima Industrial Park, 1
Takehaya, Kawashima-cho, Hashima, Gifu 501-61, Japan*

According to the ICH safety topic S1.B, "Testing for Carcinogenicity of Pharmaceuticals" (step 2 Draft Guideline), the initiation-promotion (IP) model in rodents is situated on one of the additional tests for detecting carcinogenic activity in vivo.

In this report, we examine Spironolactone (SPL), an anti-aldosterone diuretic agent and non-genotoxic carcinogen (IARC ; Lumb *et al.*), and simulate an evaluation process for carcinogenic activity using IP models, from the selection of study models to the evaluation of results and mechanistic studies concerning the action of SPL. Finally, we point out some problems associated with the IP model for assessing the carcinogenicity of novel pharmaceuticals.

1. Profile of SPL toxicity

A 4-week subacute dietary study of SPL (doses of 0, 200, 600 and 2000 ppm) using male F344/DuCrj rats was conducted to assess the maximum tolerated dose and potential target organs or tissues of carcinogenicity of SPL. Dose-dependent decreases in body weight gain were observed, and liver and thyroids weights of animals given 2000 ppm were higher than controls. Histopathologically, hepatocyte hypertro-

phy and thyroid follicular epithelial cell hypertrophy were observed at doses of 200 and 600 ppm. No other organs or tissues had histopathological changes which would suggest that SPL has carcinogenic potential.

2. Selection of assay model

The spectrum of organs and tumor types for each IP model depends on the initiator chemical(s) which were initially administered to the animals. Therefore, it is very important to predict likely target organs or tissues from other toxicology data, and to choose appropriate initiators and test systems.

A 4-weeks study suggested that the liver (hepatocytes) and thyroid (follicular epithelial cells) were potential target organs for SPL carcinogenicity, so we selected the medium-term liver bioassay for carcinogens and the thyroid IP model to assess the carcinogenic potential of SPL.

The medium-term liver bioassay for carcinogens is a well-validated test system for predicting the hepatocarcinogenicity of test substances using diethylnitrosamine-induced rat liver preneoplastic glutathione S-transferase (GST-P) positive foci (Ito *et al.*, 1988 ; Ito *et al.*, 1989). Another thyroid IP model is a typical thyroid 2-stage model which uses dihydroxypropylnitrosamine (DHPN) as an initiator. In many experiments, DHPN were used as an initiator for thyroid carcinogenesis (Hiasa *et al.* ; Moore *et al.*). In this model, carcinogenic activity is evaluated by

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Correspondence : Mamoru MUTAI at the above address.

estimating preneoplastic hyperplasia, adenoma and carcinoma of thyroid follicles.

A small scale full-range carcinogenicity study was also carried out to validate the results of these IP models.

All three studies were performed using male F344/DuCrj rats. The experimental designs and SPL doses are shown in Fig. 1.

3. Dose selection of IP models

The same dose levels of as the full-range carcinogenicity study can be used in IP models, because the same rodent species and strains as the repeated dose toxicity study were used. The above preliminary study indicated that the maximum tolerated dietary dose of SPL for male F344 rats was 2000 ppm. A dose of 2000 ppm was selected as the highest doses of the medium-term liver bioassay for carcinogens and the thyroid IP model.

4. Results of SPL carcinogenicity using IP models

Morphometrical analysis of GST-P positive foci per liver section (Fig. 2) revealed that the area of GST-P positive foci was significantly increased by SPL treatment at a dose of 2000 ppm (0.65 ± 0.39) compared to controls (0.36 ± 0.22). No significant increases were observed in the number or area of GST-P positive foci in animals given 1000 ppm or in the number of lesions at 2000 ppm. These data indicate that SPL potentially enhances the preneoplastic liver lesion development in rats at a dose of 2000 ppm. In the full-range study, hepatocellular adenomas were induced at 2000 ppm (4/24, 17% vs. 0/24

of control). Equivocal induction was seen in animals given 670 ppm (2/24, 8%). A Comparison of the results of the above liver IP model with those of the full-range study indicates that the degree of induction of GST-P positive foci in the IP model corresponds to the incidences of liver tumors in the long-term study.

The results of the thyroid IP model and the full-range study are shown in Fig. 3. Follicular hyperplasias were induced in all animals given 600 or 2000 ppm of SPL. The incidences of follicular adenomas/carcinomas were significantly higher in animals given 2000 ppm than controls. In animals given 200 ppm, no thyroid tumors were observed and the incidence of follicular hyperplasia was not significantly different from controls. These data suggest that 600 and 2000 ppm doses of SPL have the potential to induce thyroid tumors, and that 200 ppm is the putative threshold level of follicular proliferative lesions for SPL in this test system. In the full-range study, follicular adenomas/carcinomas were induced in animals given 2000 ppm of SPL. The incidences of follicular hyperplasias for 670 and 2000 ppm were significantly higher than the controls and were dose-dependent. The results of the thyroid IP model, like for liver, corresponds with those of the full-range study.

In the full-range study in male rats, no other SPL-induced tumors were observed in animals given 670 and 2000 ppm.

5. Mechanistic studies for SPL liver and thyroid carcinogenicity

To clarify the mechanisms of liver and thy-

Fig. 1. Experimental design.

Fig. 2. Quantitative results for GST-P positive foci.

Fig. 3. Incidence of thyroid follicular proliferative lesions in the thyroid IP model and the full-range study with SPL.

oids tumor promotion, we investigated hepatocyte proliferation due to BrdU (3-days continuous) labeling, and thyroxine (T4) metabolism, including T4 levels in serum and bile, and liver microsomal T4-conjugative UDP-glucuronyltransferase (UDPGT) activity. Hepatocyte BrdU labeling indices were obtained from the independent 3-day dietary study (doses: 0, 2000 ppm), and the T4 data were measured using animals of the thyroid IP model at Week 12.

The labeling indices of hepatocytes of animals given 2000 ppm SPL (5.42 ± 3.26) were 5.8-fold higher than the control value (0.93 ± 0.20). These data suggest that hepatocellular proliferation is a trigger phenomena for clarify SPL hepatocarcinogenicity. Further dose-response studies are needed to the relationship between SPL hepatocarcinogenicity and cell proliferation of hepatocytes induced by SPL.

The measurement of parameters related to T4 clearance revealed higher liver T4-UDPGT activity and higher bile T4 levels at doses of 600

Fig. 4. Effects of SPL on serum T4 levels and hepatic T4 clearance

and 2000 ppm (Fig. 4). The serum T4 levels of SPL treated animals were slightly higher than those of the controls (Fig. 4). Based on these results, we believe that SPL induced an increase in T4 excretion from liver, following which serum thyroid hormone levels probably decreased at the early phase of SPL treatment. These sequential changes caused a negative feedback mechanism via pituitary TSH release, leading to an overstimulation of the thyroid follicles. Despite the decrease in serum T4 levels, our data reveal a slight increase in T4 levels. This is because serum samples in which T4 levels were measured, were collected at Week 12 when thyroid hyperfunction had already been induced by SPL.

6. Conclusions and problems with IP models for assessing pharmaceuticals

The data from IP models generally have higher conformity with the full-range study, as shown above. IP models are useful for assessing carcinogenic potential in putative target organs or tissues suspected from the results of toxicology studies. The study size (number of animals, study period etc.) of IP models is relatively small compared with a full-range study. As was the case for the thyroid carcinogenicity of SPL, this models enable us to examine the dose-response relationship using multiple doses and the relationships between trigger changes of carcinogenicity and the induction of histopathological proliferative changes. Based on these merits, IP models may be not only valuable as an additional test, but also for assessing the carcinogenic potential of phar-

maceuticals at early stages of the development process.

The evaluation of the carcinogenicity of novel pharmaceuticals using IP models for submission to regulatory authorities is not without drawbacks. Some concerns relate to the selection and justification of IP models and an appropriate initiator, and also to the selection of a positive control which validates the results of experimental data. There are no standards for these important points. When the test substance has no suspected target organs or tissues, it is extremely important to evaluate the experimental data to justify the selection of the experimental model and the positive control substance. Others include an appropriate study design (duration of experiment and number of animals per group), the need for both sexes or not, diagnostic criteria of preneoplastic proliferative lesions etc. To use IP models to a series of carcinogenicity studies for pharmaceuticals, it is necessary to build consensuses on the above problems among ICH participants, in parallel with progress in the ICH S1.B draft guideline.

REFERENCES

- IARC (International Agency for Research on Cancer). (1980): IARC Monographs on the evaluation of carcinogenic risk of chemicals to man. WHO, IARC, Lyon, France. vol. 24, 259-273.
- Lumb, G., Newberne, P., Rust, J. H. and Wagner, B. (1978): Effects in animals of chronic administration of spironolactone: a review. *J. Environ. Pathol. Toxicol.*, **1**, 641-660.
- Ito, N., Tsuda, H., Tatematsu, M., Inoue, T., Tagawa, Y., Aoki, T., Uwagawa, S., Ogiso, M., Masui, T., Imaida, K., Fukushima, S., Asamoto, M. (1988): Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats - an approach for a new medium-term bioassay system. *Carcinogenesis*, **9**: 387-394.
- Ito, N., Imaida, K., Hasegawa, R. and Tsuda, H. (1989): Rapid bioassay methods for carcinogens and modifiers of hepatocarcinogenesis. *Crit. Rev. Toxicol.*, **19**, 385-415.
- Hiasa, Y., Ohshima, M., Kitahori, Y., Yuasa, T., Fujita, T., Iwata, C. (1982): Promoting effects of 3-amino-1,2,4-triazole on the development of thyroid tumors in rats with N-bis(2-hydroxypropyl) nitrosamine-initiated thyroid tumorigenesis in rats. *Carcinogenesis*, **3**, 381-384.
- Moore, M. A., Thamavit, W., Tsuda, H., Sato, K., Ichihara, A. and Ito, N. (1986): Modifying influence of dehydroepiandrosterone on the development of dihydroxy-di-n-propylnitrosamine-initiated lesions in the thyroid, lung and liver of F344 rats. *Carcinogenesis*, **7**, 311-316.