Short Communication

Mutagenicity of comfrey (Symphytum Officinale) in rat liver

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Comfrey is a rat liver toxin and carcinogen that has been used as a vegetable and herbal remedy by humans. In order to evaluate the mechanisms underlying its carcinogenicity, we examined the mutagenicity of comfrey in the transgenic Big Blue rat model. Our results indicate that comfrey is mutagenic in rat liver and the types of mutations induced by comfrey suggest that its tumorigenicity results from the genotoxicity of pyrrolizidine alkaloids in the plant.

British Journal of Cancer (2005) 92, 873–875. doi:10.1038/sj.bjc.6602420 www.bjcancer.com

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Keywords: comfrey; transgenic rat; cII gene; pyrrolizidine alkaloid; tandem base substitution

Comfrey (Symphytum officinale) is a tall perennial plant with large hairy leaves and small purple flowers (Winship, 1991; Betz et al., 1994). Comfrey is consumed by humans as a vegetable and a tea. It has been used as an herbal medicine for more than 2000 years to treat broken bones, tendon damage, ulcers in the gastrointestinal tract, lung congestion, and joint inflammation, and to promote wound healing (Rode, 2002). Comfrey, however, is hepatotoxic in livestock and humans and carcinogenic in experimental animals. It induced hepatic veno-occlusive disease in humans (Ridker et al., 1985; Weston et al., 1987; Bach et al., 1989; Ridker and McDermott, 1989; Yeong et al., 1990) and hepatocellular adenomas and haemangioendothelial sarcomas in rat liver (Hirono et al., 1978). Although there are no epidemiological data regarding the carcinogenicity of comfrey, these adverse effects have raised questions of its potential carcinogenicity in humans. This concern led the US Food and Drug Administration to request voluntary removal of products containing comfrey from the market in 2001 (FDA, 2001). There are presently, however, no restrictions on the use of comfrey in many parts of the world.

There is little known about the mechanism of tumour induction by comfrey. Although induction of hepatic tumours has been associated with the pyrrolizidine alkaloids (PAs) that are present in comfrey, and PAs are genotoxic and carcinogenic by binding to liver DNA in humans and animals (Prakash et al., 1999; Fu et al., 2004), a comprehensive study of comfrey mutagenesis has not been conducted. This inspired us to investigate the mutagenicity of comfrey in rat liver, a target tissue for its carcinogenesis, by using a transgenic rat mutational model (Dycaico et al., 1994).

In this study, we evaluated the mutagenicity of comfrey in the liver cII gene of Big Blue rats. The treatment schedule was based on a previous study that evaluated the carcinogenicity of comfrey (Hirono et al., 1978). Comfrey roots were obtained from Camas Prairie Products (Trout Lake, WA, USA). Pyrrolizidine alkaloids in the comfrey roots were determined by mass spectral analysis. The PAs detected were similar to those reported previously (Betz et al., 1994), and included symphytine, 7-acetyllycopsamine, and 7-acetylintermedine as major components in near equal amounts; intermedine and lycopsamine were present in relatively smaller quantity (data not shown). To determine an appropriate dose for treatment, a preliminary experiment was conducted by feeding diets containing 2, 4, and 8% comfrey. Based on a minimum effect on weight gain, lack of overt toxicity to the liver, and a maximum effect on mutagenicity, a diet containing 2% comfrey root was chosen for the mutagenesis experiment (see Supplements 1 and 2). The comfrey roots were ground and then blended with basal diet powder (NIH-31 pellets, Purina Mills International, Brentwood, MO, USA) in a Hobart Mixer to make a 2% comfrey root diet. Groups of six 6-week-old male Big Blue rats (Taconic Laboratories, Germantown, NY, USA) were fed either a basal diet or the comfrey diet. The animals were killed after 12 weeks of treatment.

Mutant frequencies (MFs) were determined for the liver cII gene of the rats treated with comfrey (Table 1). The MF for rats fed comfrey was 146 ± 15 x 10^−6, which was significantly greater than the MF for control rats, 30 ± 16 x 10^−6 (P<0.001, ANOVA, Holm–Sidak test). In the previous study of the carcinogenic activity of comfrey (Hirono et al., 1978), rats receiving a diet containing 2% comfrey root had a 42% incidence of liver tumours, while no liver tumours were found in the control rats. This correspondence between mutation induction and tumour induction suggests that comfrey induces liver tumours through a genotoxic mechanism.

The mechanisms by which the carcinogenicity and mutagenicity of comfrey are produced are not fully understood. Although we encountered no overt signs of liver toxicity in our relatively short-term study, the liver histology of rats fed comfrey for prolonged periods is quite similar to that produced by some hepatotoxic PAs (Schoental, 1968; Hirono et al., 1976, 1977). Liver cell necrosis,
Table 1  Liver cII mutant frequencies in comfrey-treated and control transgenic Big Blue rats

| Group     | Total plaques screened (× 10^3) | Mutant plaques | Mutant frequency (× 10^−4) | Mean ± s.d. (n = 6) |
|-----------|---------------------------------|----------------|-----------------------------|---------------------|
| Control   | 528                             | 15             | 28                          | 30 ± 16 × 10^−4     |
|           | 310                             | 13             | 42                          | 31 ± 14 × 10^−4     |
|           | 587                             | 8              | 14                          | 8 ± 14              |
|           | 481                             | 8              | 17                          | 8 ± 17              |
|           | 544                             | 30             | 55                          | 30 ± 55             |
|           | 339                             | 8              | 24                          | 8 ± 24              |
| Comfrey   | 254                             | 34             | 134                         | 146 ± 15 × 10^−4    |
|           | 285                             | 41             | 144                         | 41 ± 144            |
|           | 298                             | 43             | 144                         | 43 ± 144            |
|           | 288                             | 50             | 174                         | 50 ± 174            |
|           | 215                             | 32             | 149                         | 32 ± 149            |
|           | 225                             | 30             | 133                         | 30 ± 133            |

Methods for performing the cII mutagenicity assay were described previously (Mei et al., 2004). Significantly higher than the control group (P < 0.001; ANOVA, Holm–Sidak test).

Table 2  Summary of independent mutations in the liver cII gene from comfrey-treated, riddelliine-treated, and control Big Blue rats

| Type of mutation | Control | Comfrey | Riddelliine |
|------------------|---------|---------|-------------|
|                  | Number  | %       | Number      | %       | Number      | %       |
| G:C → C          | 5       | 11      | 6           | 4       | 5           | 3       |
| G:C → A          | 20      | 43      | 12          | 22      | 26          |         |
| G:C → T          | 9       | 20      | 42          | 29      | 35          |         |
| A:T → T          | 1       | 2       | 5           | 2       | 4           | 5       |
| A:T → C          | 3       | 7       | 7           | 3       | 5           | 6       |
| A:T → G          | 1       | 2       | 9           | 4       | 5           | 5       |
| Frameshift        | 7       | 15      | 26          | 13      | 8           | 10      |
| Complex           | 0       | 0       | 2           | 1       | 0           |         |
| Tandem base substitution | 0 | 0 | 33 | 17 | 7 | 8 |
| Total mutants screened | 46 | 100 | 200 | 100 | 83 | 100 |

*The mutants were sequenced using the Methods and Materials described previously (Mei et al., 2004). Spectra for comfrey- and riddelliine-treated rats are significantly different from the controls (P < 0.001; Adams and Skopek test (Adams and Skopek, 1987)): there is no significant difference between the spectra for comfrey and riddelliine (P > 0.05). †Riddelliine data are from literature (Mei et al., 2004).

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FDA (2001) FDA advises dietary supplement manufacturers to remove comfrey products from the market. USFDA, Center for Food Safety and Applied Nutrition. http://vm.cfsan.fda.gov/~dms/dspltr06.html

acknowledgements

This research was partly supported by an appointment (NM) to the Postgraduate Research Program at the NCTR administered by the Oak Ridge Institute for Science and Education.

Supplementary Information accompanies the paper on British Journal of Cancer website (http://www.nature.com/bjc).

Haemorrhage, bile duct proliferation, and liver cirrhosis are frequently encountered even in rats from experimental groups that have no tumours. This suggests that the liver tumours in comfrey-treated rats might be induced by the PAs present in comfrey. Indeed, comfrey contains up to nine PAs (Stickel and Petasites japonicus Maxim. J Natl Cancer Inst 65: 469–472

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